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THE NUTRITIVE VALUE OF ALFALFA LEAVES AND STEMS [1]

By JERRY SOTOLA [2]

Assistant professor and assistant animal husbandman, Washington Agricultural Experiment Station

INTRODUCTION

Many stockmen are convinced that the leafier as well as the greener hays possess higher nutritive value than those which are stemmy or discolored. Workers in nutrition have observed that stemmy hay, when fed alone, frequently results in excessive body losses in both calcium and phosphorus, whereas the feeding of leafier grades of hay generally results in favorable balances.

Different cuttings of alfalfa are affected by soil conditions, soil moisture, available irrigation water, temperature, relative humidity, and such other factors as age of stand, varietal differences, and differences in plant maturity. The texture also influences the curing qualities. Three cuttings made at the Washington Irrigation Branch Station at Prosser showed that the third cutting had the most leaves, and that although the stems of the first cutting were coarsest, the percentage by weight of stems was highest in the second cutting. First-cutting hay as produced under irrigation in the Yakima Valley during cool, moist weather may contain some weeds, such as cheat. The second cutting is produced during a short, hot growing period with low relative humidity. The third cutting is sometimes affected by the earliness or lateness of cold weather in the fall (*33*).

For those reasons, a separation of stems and leaves was undertaken to determine how each part of the plant functions in animal nutrition. The blossoms were included with the leaves.

PURPOSE OF THE INVESTIGATION

The experiments reported in this paper were planned to measure any existing differences in the three cuttings of alfalfa hay or their stems and leaves insofar as feeding value is concerned. Such a study should show the desirability of proper handling during the curing process, directed toward a maximum retention of those qualities important in feeding. It should further be of value in establishing grades of alfalfa hay based on true values if considered from the feeding standpoint.

The coefficients of apparent digestibility of the organic nutrients of the stems, leaves, and whole hay of the three cuttings, and the biologi-

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cal values of the proteins in the hay, in the leaves, and in the stems were determined. The gain or loss of calcium or phosphorus to lambs fed stems, leaves, or whole hay as sole rations should supply information as to their value as sources of these important minerals.

REVIEW OF LITERATURE

Headden (*17*)[3] showed that 50 percent of the weight of the alfalfa plant is leaves, and that they contain 80 percent of the crude protein and only 22 percent of the crude fiber.

Haag (*8*) showed that the limiting factor for growth of rats fed alfalfa-leaf proteins was cystine. Hart and Humphrey (*11, 12*) compared alfalfa proteins in efficiency with those of the corn grain for milk production and found these to be practically equal. Nevens (*27*) fed alfalfa proteins to rats at a 9 percent level of intake and found the biological value of these proteins to be 62. Mitchell and Hamilton (*24*), reviewing the work of Hamilton, Nevens, and Grindley, show that of the total nitrogen in alfalfa hay, 8 percent is arginine, 3.9 percent histidine, 4.4 percent lysine, 1 percent cystine, and 19.1 percent is nonprotein nitrogen.

Hart et al. (*14*) observed that a more liberal storage of calcium was obtained when liberally milking cows were on fresh green alfalfa than when they were on dry alfalfa. Steenbock et al. (*39*) showed that hay cured in the field and kept under caps produced calcium equilibrium when fed to cows in full milk flow, in quantities of 10 pounds, in addition to corn silage and grain. Hay cured in the windrow for 4 days with exposure to air and light resulted in negative calcium balances when fed in the same manner. Smith and Briggs (*35*) have shown that alfalfa cured in the dark is deficient in antirachitic potency and that synthesis of vitamin D occurs when the alfalfa is exposed to sunlight in the curing process. Alfalfa carefully spread out on the field from 11:15 a.m. one day to 12 m. the next, during which time the sun shone 15 hours and 5 minutes, possessed mild calcifying powers. The hay which lay in the swath for 1 week and was exposed to sunshine for 57.3 hours and to 0.37 inches of rain was found to be highly antirachitic.

Russell (*32*) showed that the Mason process employing artificial heat produced dried alfalfa leaves with a vitamin A potency seven times greater than that of the same product cured in the field. However, the leaves when cured artificially contained only small quantities of the antirachitic vitamin. When the alfalfa was dried in the sun, without exposure to dew or rain, there was an increase in the antirachitic potency of the leaves, but a decrease in vitamin A content.

Bethke and Kick (*4*) report that exposure of alfalfa hay to the sun, rain, or dew over a period of several days resulted in a marked loss of vitamin A. Hauge and Aitkenhead (*16*) consider vitamin A to be probably the most important single nutritive factor in alfalfa that may be affected by curing.

Hathaway, Davis, and Graves (*15*) showed that under the conditions of their experiment artificially cured alfalfa was twice as potent in vitamin A as was the field-cured hay.

Recent work by Smith and Briggs (*34*) on the vitamin A content of alfalfa leaves shows that a loss of 20 to 33 percent in this factor occurs by allowing the alfalfa to be spread out carefully for 2¾ hours,

[3] Reference is made by number (italic) to Literature Cited, p. 942.

from 11:15 a.m. to 2 p.m. No greater degree of destruction occurred when the time of exposure was increased to 6¾ hours in the same day. No loss of green color in the leaves could be observed in comparison with leaves cured in the dark, with which the other samples were compared in respect to vitamin A potency.

The same workers show that a 75-percent loss of vitamin A results when the alfalfa is allowed to lie in the field under the same conditions overnight and until 8 a.m. the next day. This loss was increased to 84 percent upon further exposure until 12 m. the second day. Alfalfa severely bleached as a result of 1 week's exposure to sun and rain in the field while in the swath retained but 4 percent of the vitamin A present, as compared with the sample cured in the dark.

Douglas, Tobiska, and Vail (*5*) working under Colorado conditions found that hay cut at the early bloom stage contained more vitamin A than did that cut at other stages of growth, the method of curing being the same. The hay cured in diffused light was superior in vitamin A content in most cases to that cured in direct sunlight.

Douglas, Tobiska, and Vail (*5*) also found that second-cutting hay was lower in vitamins B and G than that of the first or third cuttings. When the third crop reached the early bloom stage before frost, it usually rated second in nutritive value. The inferiority of the second crop was attributed to the heat of the summer, with excessive transpiration and woodiness of plant tissue.

In the above studies rats were used as the experimental animals. In another phase of the work, in which guinea pigs were used, it was shown that green alfalfa contains an abundance of vitamin C, practically all of which was lost when the plants were cured for hay.

Hunt et al. (*19*) point out that hays vary in their "quality-producing" factors, depending upon the time of harvesting and method of curing. Vitamin B is present in such small amounts in hays as to be of small importance. A high protein and a high vitamin G content are correlated in a general way with a good green color and leafiness. As the greenness fades, due to the stage of maturity of the plant, the protein and vitamin G content also decrease while the fiber increases.

Meigs, Hartman, and Converse[4] recently have pointed out a tendency toward parallelism between vitamin A content and color in both alfalfa hay and butter. The color of butter seemed to be more influenced by feed than by the breed of the cow.

Meigs and Converse (*22*) stress good quality hay for best milk production and reproduction of dairy cows.

That quality of hay may be influenced by the curing processes employed is clearly shown by Willard (*42*) and Westover (*41*) who stated that alfalfa leaves do not assist in withdrawing moisture from the stems in the curing process, thus supporting the view of Higgins (*18*). Alfalfa apparently cures at least as rapidly with the leaves removed as with them attached.

Kiesselbach and Anderson (*20, 21*) state that the supposed transpiratory function of the leaves in alfalfa severed from its roots is a negligible factor in the curing process. Prolonged curing in the swath resulted in loss of leaves through shattering and also produced bleaching, while windrowing and cocking relatively green plants

[4] MEIGS, E. B., HARTMAN, A. M., and CONVERSE, H. T. VITAMIN A AND COLOR IN HAY AND MILK. U.S Dept.Agr., Bur. Dairy Indus., 4 pp. 1933. [Mimeographed.]

materially extended the curing period. Judicious partial swath curing to hasten the rate of drying without bleaching followed by windrowing and by prompt storage when cured, seemed the best practices.

Higgins (*18*) showed that during clear hot weather, alfalfa dried in the swath to a moisture content of approximately 25 percent was badly bleached and that the leaves were very brittle, a large proportion of them being lost. Hay dried in the swath lost 25.3 percent in weight when subjected to shaking as compared to losses of 20.7, 21.6, 22.3, and 16.6 percent for hay dried in the swath in the shade, in the windrow and not turned, in the windrow and turned, and that dried in the cock, respectively. The ordinary mechanical loss of leaves in field curing alfalfa hay is placed by Headden (*17*) at 15 to 20 percent.

Rather (*30*) stressed the importance of getting the hay out of the swath and into the windrow within a few hours after it was cut. Willard (*42*) and Westover (*41*) supported this view, and showed that bleaching and loss of leaves by shattering and crumbling were thereby reduced.

Headden (*17*) stated that overcuring resulted in the loss of leaves and stems, amounting to one fifth to two thirds of the crop.

Kiesselbach and Anderson (*20, 21*) and Parker (*28*) stressed purity, texture, and high percentage of leaves, clinging foliage, green color, and pliable stems as essentials of high-quality hay, whereas Rather (*30*) emphasized green color, pleasant aroma, and retention of leaves.

Haag and his coworkers (*10*) explain the negative nitrogen balances obtained when alfalfa hay alone was fed to dairy cattle, as due to a faulty energy intake on account of bulk, a low biological value of the proteins, and a low phosphorus content in turn supposedly related to protein metabolism. For dairy cows a sole-alfalfa-hay ration does not seem to supply adequate phosphorus and sufficient total digestible nutrients, except for moderate milk production.

The calcium-phosphorus ratio of alfalfa is reported to vary from 3 to 1, up to 25 to 1. The work of Meigs, cited by Haag (*8*), favors a 2 to 1 calcium-phosphorus ratio, and later a 1.5 to 1 ratio. Haag and his coworkers (*9*) in a later paper state that apparently the storage of calcium is conditioned by the relatively low phosphorus intake.

Woll (*43*) cites instance in which dairy cows, beef cattle, sheep, and even hogs frequently receive nothing but alfalfa throughout the year, either as pasture or soilage in the summer, or as hay in the winter. Apparently no ill effects resulted from this monotonous diet.

METHOD OF EXPERIMENTATION

PLAN OF THE WORK

Three cuttings of northern-grown common alfalfa were studied by feeding the stems, whole hay, and leaves of each cutting to a different group of six lambs. Owing to the length of time, approximately 100 days, that each of the six lambs was confined to experimental feeding of each cutting of hay, it was possible to study only one cutting each year to avoid feeding old hay. The following order was followed with each cutting:

1. Preliminary period
2. Nearly nitrogen-free ration

3. Preliminary period
4. Stems of alfalfa hay
5. Preliminary period
6. Whole alfalfa hay
7. Preliminary period
8. Leaves of alfalfa hay
9. Preliminary period
10. Nearly nitrogen-free ration

Experimental periods of 10 days (*36*, *38*) were preceded by preliminary periods of 10 days. In view of the nearly nitrogen-free feeding, periods 1 and 9 were of 20 days duration to accustom the lambs to the unusual rations. During all experimental tests the lambs were confined in specially constructed metabolism crates (*36*).

Stems and leaves, including blossoms, were separated by hand from the first, second, and third cuttings. Approximately 500 pounds each of stems, leaves, and blossoms, and of whole alfalfa hay were used. The stems and hay were fed chopped.

METHOD OF SAMPLING AND ANALYSIS

The samples of feed were thoroughly mixed, and samples were then taken for chemical study. The feces and urine were treated by the method of French (*7*) to prevent loss of nitrogen. Official methods (*3*) of analysis were used.

HAY SAMPLES USED

In 1929 first-cutting alfalfa at the half-bloom stage (*36*) was placed in a barn loft while still green within a 2-hour period after cutting and cured slowly in subdued light. In November, the leaves, including the blossoms, were separated from the stems. Representative samples for chemical anaylsis were taken. In 1930 a sample was taken from the second cutting and separated into stems and leaves. The following year the third cutting was handled similarly. These were cured under the same conditions as the first cutting.

The results of the mechanical separations expressed in percent, on the basis of the weight of air-dried hay, are shown in table 1.

The first and third cuttings, according to the official hay grades (*29*), would rate as U.S. No. 1 extra leafy, and the second cutting as U.S. No. 1 alfalfa on the basis of leafiness. Although no color charts were used, all of the hay samples, because of the method of curing, would have graded as extra green.

TABLE 1.—*Percentage of stems and leaves in the alfalfa hay studied*

Cutting	Leaves	Stems
	Percent	*Percent*
First	50.27	49.73
Second	45.82	54.18
Third	65.61	34.39

LAMBS AND THEIR BODY WEIGHTS

A different group of six cut-back range lambs was used each year. The initial and final weights are reported in table 11. Each year the lambs were selected in pairs, one pair being light, another medium,

and a third fairly heavy in weight. As far as could be determined, the lambs in all groups were sired by Hampshire rams and out of Lincoln-Merino crossbred ewes.

In 1929 there were 5 ewe lambs and 7 wethers in the group; in 1930 only wethers were used; and in 1931, 7 ewes and 5 wethers were used. The individual records make it possible to trace sex differences. Losses in weight by lambs during periods of nearly nitrogen-free feeding were the rule. When stems were fed most lambs lost weight although some gained, and a few showed no change. On whole-hay rations the lambs gained weight except on first-cutting hay. On the leaf rations gains and losses alternated in the first- and second-cutting hay. On the third-cutting rations losses were the rule. Weight fluctuations of lambs during 10-day periods when the animals had access to water at all times are of slight significance, especially when based on single weighings.

RATIONS FED

Along with the nearly nitrogen-free rations, each lamb was fed 30 g of mixed oil daily. The mixture consisted of 1 part cod-liver oil and 3 parts raw linseed oil.

The nearly nitrogen-free mixture contained 460 parts of cut mature Albit wheat straw low in nitrogen, 250 parts of cane sugar, 250 parts of powdered cornstarch, 10 parts calcium carbonate (c.p.) and 10 parts sodium chloride. The dry matter consumed by each lamb during each 10-day period is shown in table 11. The oil was used to raise the caloric value of the ration, so that even during days of low dry-matter intake the energy value of the nearly nitrogen-free ration might be well above the maintenance requirement. This condition must be met so that the fecal and urinary nitrogen excreted during periods of nearly nitrogen-free feeding may be strictly of the minimum endogenous type.

The quantity of roughage consumed is according to Armsby (*2*) not a factor in digestibility. The dry matter consumed in the form of stems, whole hay, and leaves was fairly constant in 1929, good for stems and leaves but high for whole hay in 1930, almost identical to 1930 figures for whole hay and leaves, but low for stems in 1931. In all cases the lambs were fed these feeds according to their appetites.

ANALYTICAL PROCEDURE

The Gunning modification of the Kjeldahl method was used in determining nitrogen (*3*). The calcium and phosphorus were determined by official volumetric methods. Special precautions were taken to prepare the samples properly (*6*).

CHEMICAL COMPOSITION OF ALFALFA HAY SAMPLES

The chemical composition of the stems, whole hay, and leaves of the first, second, and third, cuttings of alfalfa hay are given in table 2. Figures for moisture just as the feed was fed to the lambs in the metabolism crates are given, too. A better comparison can be made if the figures are reduced to a common moisture basis, 10 percent being considered suitable. Such figures are also contained in table 2.

The stems contained more crude protein than is generally supposed, the percentage decreasing from 8.58 in the first to 7.63 in the third cutting. The crude fiber content was high, ranging from 42.83 to 39.74 percent.

TABLE 2.—*Chemical composition of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa*

Part of plant and cutting	Year	Moisture content as fed	Composition on a 10-percent moisture basis							
			Protein (N×6.25)	Ash	Crude fat	Crude fiber	Nitrogen-free extract	Calcium	Phosphorus	P:Ca ratio
Stems:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
First	1929	11.84	8.58	4.83	1.05	42.20	33.34	0.755	0.106	1:7.1
Second	1930	12.68	8.29	5.80	1.63	42.83	31.45	.780	.188	1:4.2
Third	1931	13.66	7.63	5.62	1.23	39.74	35.78	1.042	.096	1:10.8
Whole hay:										
First	1929	12.19	11.96	6.40	1.46	34.54	35.64	1.447	.135	1:10.7
Second	1930	13.92	14.05	7.96	2.02	36.37	29.60	1.492	.229	1:6.5
Third	1931	18.54	15.81	7.81	2.83	23.53	40.02	2.580	.166	1:15.6
Leaves:										
First	1929	13.43	18.31	9.34	2.52	18.01	41.82	2.953	.188	1:15.7
Second	1930	12.54	19.98	9.79	3.12	15.61	41.50	2.286	.281	1:8.1
Third	1931	15.05	19.33	9.16	3.38	15.80	42.33	3.053	.191	1:16.0

In comparing the data for the whole hay with those of ordinary alfalfa hays, it should be kept in mind that the sample studied was not field cured, and that it contained practically all the upper portion of the plant, there being no leaf loss such as occurs in field curing. The percentage of protein increased from the first to the third cutting, the value for the third, 15.81 percent, being especially high. Leafiness of the third-cutting hay was reflected by its low fiber content, 23.53 percent, as compared with 34.54 and 36.37 percent, respectively, for the first and second cuttings.

The leaves contained the highest percentage of protein, which ranged from nearly 20 to 18.31 percent. The crude fiber in the leaves ranged from 15.61 to 18.01 percent. This percentage may of course be affected somewhat by the thoroughness of the mechanical separation.

The calcium and phosphorus in the stems and leaves afforded an interesting comparison. The leaves of the first cutting contained almost four times as much calcium as the stems, but the difference in phosphorus content was not so great.

Ames and Boltz (*1*) have found 50 percent of the total phosphorus of the alfalfa plant to be in the organic form. While the amount of total phosphorus was found by them to be greater in the first cutting, a greater proportion of it was combined as organic phosphorus in the second cutting.

The relationship of percentages of calcium in stems to that in leaves was only 1 to 3 for the second and third cuttings. The ratio of phosphorus in stems to that in leaves was 1 to 2 in the first and third cuttings and 2 to 3 in the second cutting.

The preceding data become more effective if the percentage distribution of nutrients in the stems and leaves per 100 pounds of hay is computed. Such data for the first, second, and third cuttings are shown in table 3.

It was observed that 67 to 83 percent of the protein was found in the leaves. Therefore, the higher the percentage of leaves in the hay, the higher will be its protein content. This conforms with the results of other workers.

TABLE 3.—*Distribution of nutrients (as percentage of total) per 100 pounds in stems and leaves of the first, second, and third cuttings of alfalfa*

[10-percent moisture basis]

Cutting	Protein (N×6.25)	Ash	Fat	Crude fiber	Nitrogen-free extract	Calcium	Phosphorus
Stems: [a]							
First	31.67	33.84	29.18	69.86	44.09	20.18	35.89
Second	32.91	41.19	38.18	76.43	47.26	28.75	54.20
Third	17.14	24.33	16.02	56.86	30.69	15.17	20.91
Leaves: [a]							
First	68.33	66.16	70.82	30.14	55.91	79.82	64.11
Second	67.09	58.81	61.82	23.57	52.74	71.25	45.80
Third	82.86	75.67	83.98	43.14	69.31	84.83	79.09

[a] First cutting of hay contained 49.73 percent of stems by weight and 50.27 percent of leaves, the second cutting 54.18 percent of stems and 45.82 percent of leaves, and the third cutting 34.39 percent of stems and 65.61 percent of leaves.

Ames and Boltz (*1*) report that 80 percent of the protein in the alfalfa plant is present in the leaves, and that under favorable circumstances the loss of leaves may be 15 percent of the total crop. The nitrogen content of alfalfa was found to be less in the second cutting than in the first, and increased in the third.

The data in table 3 were secured by multiplying the percentages of stems and leaves in the various cuttings by the figures of their respective composition. Obviously from the standpoint of mineral nutrition, the leaves are the most liberal source of calcium and phosphorus.

EFFECT OF ALFALFA LEAVES AND STEMS ON WATER CONSUMPTION AND THE URINARY AND FECAL EXCRETION OF LAMBS

The rations fed, consisting either of alfalfa stems, leaves, or whole hay, afforded an excellent opportunity of studying their effect on water consumption and on fecal and urinary excretions of growing lambs.

The data in table 4 show the diuretic effect as well as the thirst-stimulating qualities of alfalfa leaves.

The lambs consumed 2.12 times as much water as of feed when fed stems of the first cutting, the values for the second and third cuttings being 3.07 and 2.33 times the quantity of feed eaten.

The leaves of first, second, and third cuttings required 4.09, 3.95, and 2.00 times their weight in water. The whole hay ranked intermediate. It is recognized that the external temperature affects water consumption. The feces were most bulky when stems were fed.

The stems, whole hay, and leaves, in order, exerted progressively greater effects on urine excretion. The leaves, on the average, stimulated three and one third times as much urine excretion as the stems.

Leaf rations resulted in urine richer in nitrogen than when stems were fed. The urine of lambs fed leaves of the first cutting contained a total of 2.75 times as much as when stems were fed.

TABLE 4.—*Water consumption and feces and urine excreted per head by 18 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa as their sole ration*

Cutting and trial no.	Ration	Total feed eaten	Total dry matter consumed	Total water consumed	Water consumed per gram of food	Total feces	Feces per 100 grams of food consumed	Feces per gram of dry matter
First:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
102 and 103	Stems	6,537.5	5,763.3	13,944.2	2.12	7,756.2	118.37	1.34
104 and 105	Whole hay	5,695.0	5,001.0	14,536.3	2.54	3,731.2	65.60	.75
106 and 107	Leaves	6,191.7	5,359.8	25,260.2	4.09	2,609.2	42.21	.49
Second:								
501	Stems	6,229.2	5,721.3	19,423.8	3.11	7,137.2	114.02	1.24
502	Whole hay	8,720.8	7,939.5	27,338.3	3.13	5,993.0	69.00	.76
503	Leaves	6,500.0	6,014.5	25,537.5	3.95	2,535.7	39.40	.43
Third:								
1001	Stems	4,949.0	4,273.5	11,517.2	2.33	7,557.3	152.85	1.76
1002	Whole hay	7,843.3	6,387.0	17,506.8	2.22	5,054.0	64.50	.79
1003	Leaves	7,486.7	6,359.5	15,070.0	2.00	3,251.2	43.43	.52

Cutting and trial no.	Ration	Total volume of urine	Urine per gram of dry matter consumed	Nitrogen in urine	Nitrogen in feces	Nitrogen voided—	
						In urine	In feces
		Cc	*Cc*	*Grams*	*Grams*	*Percent*	*Percent*
First:	Stems	5,096.7	.88	38.28	43.67	46.7±2.64	53.3±2.64
102 and 103	Whole hay	11,914.2	2.36	81.81	36.93	68.9±2.08	31.1±2.08
104 and 105	Leaves	17,444.5	3.26	105.13	44.28	70.4± .87	29.6± .87
106 and 107							
Second:							
501	Stems	3,215.8	.56	28.70	40.87	41.3±2.90	58.7±2.90
502	Whole hay	11,300.0	1.41	77.52	59.40	56.6±1.27	43.4±1.27
503	Leaves	17,253.3	2.87	156.12	43.20	78.3± .46	21.7± .46
Third:							
1001	Stems	3,520.5	.82	28.27	28.27	50.0±3.20	50.0±3.20
1002	Whole hay	8,530.0	1.33	89.08	46.74	65.6± .50	34.4± .50
1003	Leaves	8,585.1	1.35	131.73	48.79	73.0± .47	27.0± .47

Of further interest was the partition of total excretory nitrogen, between the urine and feces. Whether the rations of leaves and stems result in a greater distribution of excretory nitrogen in the feces or urine has partly been answered above. In the case of the stem rations the trend was toward an almost equal partition of the total excretory nitrogen between the urine and feces. For the rations of whole hay, with the exception of the second cutting, approximately 67 percent of the nitrogen excreted was found in the urine. There was no question that the shift of excretory nitrogen was toward the urine when leaves were fed. Since the leaves contributed more digestible nitrogen than did the stems, and since the lamb can use only so much nitrogen at a time, it was logical to find more nitrogen in the urine when leaf rations were fed. This may be directly attributed to the deamination of surplus amino acids.

CHEMICAL COMPOSITION OF THE FECES

A striking variation in the composition of sheep feces as affected by the type of feed is shown in table 5.

The dry matter content of the feces was highest when alfalfa leaves were fed. In fact, the ration exerting the highest diuretic effect resulted in feces of the lowest moisture content.

TABLE 5.—*Average chemical composition (percent) of feces of 18 lambs fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa*

Part of plant and cutting	Dry matter	Protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract
Stems:						
First	42.22	3.59	22.43	0.37	2.85	12.98
Second	41.98	3.66	22.74	.62	3.55	11.41
Third	30.07	2.30	15.28	.55	2.97	8.97
Whole hay:						
First	63.20	6.21	29.18	1.90	6.37	19.54
Second	59.48	6.23	28.62	1.98	6.89	15.76
Third	49.90	5.81	19.18	2.86	9.26	12.79
Leaves:						
First	80.26	10.71	18.34	5.34	17.03	28.84
Second	71.65	10.64	19.30	5.32	12.64	23.75
Third	63.14	9.42	14.44	5.61	13.65	20.02

Feces from lambs fed alfalfa stems contained only 2.85 to 3.55 percent of ash, whereas when leaves were fed the ash varied from 12.65 to 17.03 percent. The leafier grades of hay fed to lambs had a higher fertilizer value.

COEFFICIENTS OF APPARENT DIGESTIBILITY

The usual procedure, as outlined previously (*36*) was followed in determining the coefficients of apparent digestibility. Table 6 contains such coefficients for the first, second, and third cuttings of hay.

TABLE 6.—*Coefficients of apparent digestibility of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods*

FIRST CUTTING

Part of plant and trial no.	Lamb no.	Dry matter	Crude protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract
Stems:							
102	100	46	55	39	51	41	55
	101	46	52	40	74	39	55
	102	45	52	39	50	29	54
103	103	46	56	36	62	35	59
	104	40	40	32	56	12	56
	105	43	49	35	57	23	56
Average		44.3	50.7	36.8	58.3	29.8	55.8
Whole hay:							
104	100	53	67	44	19	34	65
	101	59	69	52	.....	40	70
	102	53	64	44	12	31	65
105	103	51	64	40	15	32	65
	104	50	63	39	12	34	62
	105	51	64	41	14	30	64
Average		52.8	65.2	43.3	14.4	33.5	65.2
Leaves:							
106	100	64	78	49	11	34	76
	101	63	76	84	.....	26	62
	102	51	69	38	.....	8	64
107	103	65	77	56	17	13	80
	104	62	75	53	72	22	74
	105	63	74	53	19	25	75
Average		61.3	74.8	55.5	29.8	21.3	71.8

TABLE 6.—*Coefficients of apparent digestibility of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods*—Continued

SECOND CUTTING

Part of plant and trial no.	Lamb no.	Dry matter	Crude protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract
Stems:							
501	201	50	56	41	56	41	60
	202	45	54	35	57	45	56
	207	50	57	45	58	27	62
	204	53	57	45	54	41	64
	205	43	41	37	52	13	56
	206	48	40	43	59	21	61
Average		48.2	50.8	41.0	56.0	31.3	59.8
Whole hay:							
502	201	60	72	34	38	43	73
	202	55	70	32	33	48	70
	207	57	72	35	29	45	73
	204	55	71	36	33	35	70
	205	54	68	36	26	34	70
	206	52	66	33	18	41	67
Average		55.5	69.8	34.3	29.5	41.0	70.5
Leaves:							
503	201	68	80	47	55	52	75
	202	66	79	45	40	48	74
	207	72	81	57	35	53	80
	204	71	80	57	29	50	80
	205	69	78	53	28	49	78
	206	72	81	58	28	54	80
Average		69.7	79.8	52.8	35.8	51.0	77.8

THIRD CUTTING

Stems:							
1001	1	50	70	42	26	19	61
	2	50	58	41	34	29	61
	3	48	53	40	28	19	60
	4	44	28	38	22	----	63
	5	46	49	38	30	16	59
	6	46	53	36	36	24	62
Average		47.3	51.8	39.2	29.3	21.4	61.0
Whole hay:							
1002	1	60	73	41	19	17	77
	2	60	73	43	23	16	77
	3	61	74	42	29	17	78
	4	60	74	42	29	11	77
	5	60	74	42	36	13	76
	6	62	76	44	33	18	78
Average		60.5	74.0	42.3	28.2	15.3	77.2
Leaves:							
1003	1	66	76	58	17	32	76
	2	68	78	59	20	36	78
	3	69	78	57	32	35	79
	4	68	78	58	24	23	80
	5	66	76	57	17	28	77
	6	70	79	60	31	35	79
Average		67.8	77.5	58.2	23.5	31.5	78.2

The dry matter of the stems of the first-cutting hay was 16 percent less and that of the leaves 16 percent more digestible than that of the whole hay. The protein was 22 percent less digestible in the stems and 15 percent more digestible in the leaves than in the whole hay.

A similar comparison for fiber and nitrogen-free extract showed the nutrients of the stems to be less, and the nutrients of the leaves to be more digestible than the corresponding nutrients of the whole hay.

Table 6 also shows that in the second cutting, the dry matter of the stems was 13 percent less and the dry matter of the leaves 26 percent more digestible than that of the whole hay. The protein of the stems was 27 percent less digestible and that of the leaves 14 percent more digestible.

The results secured in feeding the third-cutting hay (table 6) again showed the dry matter of the stems to be 22 percent less, and of the leaves 12 percent more digestible than that of the whole hay. A big difference in digestibility between the protein of the stems and of the whole hay was noticed, the stem protein in the third cutting being 30 percent less digestible than that in the whole hay.

The results with each of the 3 cuttings were arranged in table 7 and averaged. The average coefficients of 18 determinations for stems, whole hay, and leaves for all three cuttings were also computed.

TABLE 7.—*Coefficients of apparent digestibility of stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to lambs*

Part of plant and cutting	Dry matter	Crude protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract
Stems:						
First	44.3	50.7	36.8	58.3	29.8	55.8
Second	48.2	50.8	41.0	56.0	31.3	59.8
Third	47.3	51.8	39.2	29.3	21.4	61.0
Average	46.6	51.1	39.0	47.9	27.5	58.9
Whole hay:						
First	52.8	65.2	43.3	14.4	33.5	65.2
Second	55.5	69.8	34.3	29.5	41.0	70.5
Third	60.5	74.0	42.3	28.2	15.3	77.2
Average	56.3	69.7	40.0	24.0	29.9	71.0
Leaves:						
First	61.3	74.8	55.5	29.8	21.3	71.8
Second	69.7	79.8	52.8	35.8	51.0	77.8
Third	67.8	77.5	58.2	23.5	31.5	78.2
Average	66.3	77.4	55.5	29.7	34.6	75.9

The stems of the second cutting excelled in digestibility of dry matter, and the stems of the third cutting in protein. The fiber of the second-cutting stems was most digestible, as was the nitrogen-free extract of the stems of the third cutting.

At the Illinois Experiment Station (*25*) 12 head of lambs weighing from 96.4 to 104 pounds were placed upon a maintenance allowance of alfalfa hay. The coefficients for the nutrients in the alfalfa hay were as follows: Dry substance 55.6, crude protein 67.3, nitrogen-free extract 71, ether extract 19, and crude fiber 26.3. Later work at the same station (*26*) with 4-year-old ewes fed alfalfa hay and approximately 0.15 pound of linseed meal per head showed that the average digestibility of the nutrients in the hay alone were as follows: Dry substance 64.3, crude protein 73, nitrogen-free extract 78, crude fiber 43, and ether extract 51.7. These results are higher than in the case where alfalfa was fed alone and may be attributed largely to the associative effect of the linseed meal.

These results in the main conform with those of the work reported above.

DIGESTIBLE NUTRIENTS IN ALFALFA STEMS, WHOLE HAY, AND LEAVES OF THE FIRST, SECOND, AND THIRD CUTTINGS

The coefficients of apparent digestibility are of practical significance only when used in determining the digestible nutrients of the stems, leaves, and whole hay.

Such data in table 8 show a wide difference in the stems, whole hay, and leaves. In whole numbers there was 42 percent digestible dry matter and 4 percent of digestible protein in alfalfa stems. Third-cutting hay excelled others in digestible protein. The difference between the protein of the stems of the first and second cutting was small. The stems of second-cutting hay contained the largest percentage of digestible crude fiber and of total digestible nutrients, while the stems of the third cutting contained most nitrogen-free extract. Averages for the stems of all three cuttings are included.

TABLE 8.—*Percentage of digestible nutrients in stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to animals*

[10-percent moisture basis]

Part of plant and cutting	Dry matter	Protein (N×6.25)	Crude fiber	Fat	Ash	Nitrogen-free extract	Total digestible nutrients	Nutritive ratio
Stems:								
First	39.87	4.35	15.53	0.61	1.44	18.60	39.85	1:8.16
Second	43.38	4.21	17.56	.91	1.82	18.81	42.63	1:9.13
Third	42.60	3.95	15.57	.36	1.20	21.83	42.16	1:9.67
Average	41.95	4.17	16.22	.63	1.49	19.75	41.55	1:8.96
Whole hay:								
First	47.52	7.80	14.96	.21	2.14	23.24	46.47	1:4.96
Second	49.95	9.81	12.47	.60	3.26	20.87	44.50	1:3.54
Third	54.45	11.70	9.96	.80	1.20	30.87	54.33	1:3.64
Average	50.64	9.77	12.46	.54	2.20	24.99	48.43	1:3.96
Leaves:								
First	55.17	13.70	10.00	.75	1.99	30.03	55.42	1:3.05
Second	62.73	15.94	8.24	1.12	4.99	32.29	58.99	1:2.70
Third	61.05	14.98	9.19	.79	2.89	33.09	59.04	1:2.94
Average	59.65	14.87	9.14	.89	3.29	31.80	57.82	1:2.89

Third-cutting hay excelled the others in the percentage of digestible dry matter, protein, nitrogen-free extract, and total digestible nutrients. This was due largely to its extreme leafiness, which was 65.61 percent. The leafiness was further reflected in a low content of digestible fiber, these two factors being inversely related.

The second-cutting hay was slightly superior to the first cutting in its content of digestible dry matter and protein. The first-cutting hay, however, was higher than the second cutting in digestible crude fiber, nitrogen-free extract, and total digestible nutrients.

Leaves of the second cutting contained the most digestible dry matter and protein. The difference between the total digestible nutrients in the second and first cutting was 6.5 percent in favor of the second cutting.

Averages of all the stems of the three cuttings and of all the leaves, when compared with each other, showed large differences.

A given weight of leaves was almost 3.57 times as efficient as the same weight of stems in supplying digestible protein, and the leaves were less bulky. The leafier the hay, the better source of digestible protein it becomes, irrespective of cutting.

The leaves contained almost a third more per pound of total digestible nutrients than did the stems.

An interesting comparison resulted when the values for the digestible protein in the stems and leaves of the three cuttings were applied to the percentage of stems and leaves in each of the three cuttings. Such data show that 76 percent of the digestible protein in the first and second cuttings and 88 percent of the third cutting was contained in the leaves. This, of course, was on the basis of the samples as produced at the Washington Irrigation Branch Station.

The work of Hart, Kline, and Humphrey (*13*) is interesting in this connection. They studied the availability of the dry matter, protein, and calcium in second-cutting alfalfa hay fed to heavy-milking cows. The treatment of the hay was as follows: (1) Dried immediately in a Koon artificial drier; (2) partly dried in the field and then dried in the artificial drier; and (3) completely dried in the field.

The availability of dry matter, protein, and calcium was much alike in the three samples of hay. The high temperature of 480° to 535° C. for 40 seconds to which sample 1 was subjected did not reduce the availability of the nutrients under observation.

NITROGEN METABOLISM STUDIES

The gain or loss of protein can be detected readily by a comparison of the total feed nitrogen and that in the excreta. The values obtained in these trials are given in table 9 and summarized in table 10. A positive balance of nitrogen was found in every period during which alfalfa leaves were fed, irrespective of whether the leaves came from the first-, second-, or third-cutting hay. The largest retention of nitrogen during any 10-day period of leaf feeding was 47.5 g for lamb 3 in trial 1003 fed third-cutting leaves, and the smallest, 12.49 for lamb 3 during trial 106, when leaves of the first cutting were fed. Obviously the best single retention of nitrogen on whole hay occurred when alfalfa of the second cutting was fed, a balance of 68.8 g of nitrogen for lamb 2 in trial 502 being obtained.

TABLE 9.—*Nitrogen balance of 6 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa during 10-day periods*

FIRST CUTTING

Part of plant and trial no.	Lamb no.	Feed eaten	Nitrogen consumed	Nitrogen voided in—			Nitrogen balance	Intake stored
				Feces	Urine	Total outgo		
Stems:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
102	1	5,925	79.73	36.07	37.55	73.62	+6.11	7.66
	2	6,525	87.83	42.10	51.83	93.93	−6.10	
103	3	6,525	87.80	42.29	29.47	71.76	+16.04	18.27
	4	6,750	90.83	40.25	50.27	90.52	+.31	.34
	5	6,750	90.83	54.91	28.99	83.90	+6.93	7.63
	6	6,750	90.83	46.43	31.58	78.01	+12.82	14.11
Average		6,537.5	87.97	43.68	38.28	81.96	+6.01	6.83
Whole hay:								
104	1	6,000	112.03	37.25	145.21	182.46	−70.43	
	2	6,000	112.03	34.43	80.80	115.23	−3.20	
	3	6,000	112.03	40.29	65.96	106.25	+5.78	5.16
105	4	5,390	100.64	35.96	72.65	108.61	−7.97	
	5	5,390	100.64	37.01	59.01	96.02	+4.62	4.59
	6	5,390	100.64	36.62	67.26	103.88	−3.24	
Average		5,695.0	106.34	36.93	81.82	118.75	−12.41	
Leaves:								
106	1	6,000	169.06	37.66	102.59	140.25	+28.81	17.04
	2	6,000	169.06	40.66	108.90	149.56	+19.50	11.53
	3	6,000	169.06	53.66	103.91	156.57	+12.49	7.39
107	4	6,400	180.33	41.61	110.59	152.20	+28.13	15.60
	5	6,350	178.92	45.33	98.43	143.76	+35.16	19.65
	6	6,400	180.33	47.74	106.11	153.85	+26.48	14.68
Average		6,191.7	174.46	44.28	105.09	149.37	+25.09	[a] 14.38

SECOND CUTING

Stems:								
501	1	7,275	98.43	43.33	36.34	79.67	+18.76	19.06
	2	5,100	69.00	31.60	42.54	74.14	−5.14	
	3	8,550	115.68	49.57	44.16	93.73	+21.95	18.97
	4	5,600	75.77	32.88	44.36	77.24	−1.47	
	5	5,425	73.40	43.58	28.15	71.73	+1.67	2.27
	6	5,425	73.40	44.28	37.84	82.12	−8.72	
Average		6,229.2	84.28	40.87	38.90	79.77	+4.51	[a] 5.35
Whole hay:								
502	1	9,000	204.66	57.38	89.08	146.46	+58.20	28.44
	2	8,225	187.04	55.58	62.63	118.21	+68.83	36.80
	3	10,425	237.06	65.75	130.57	196.32	+40.74	17.19
	4	8,225	187.04	55.19	81.93	137.12	+49.92	26.69
	5	8,225	187.04	59.74	81.46	141.20	+45.84	24.51
	6	8,225	187.04	62.78	81.88	144.66	+42.38	22.66
Average		8,720.8	198.31	59.40	87.93	147.33	+50.98	[a] 25.71
Leaves:								
503	1	6,000	197.53	40.16	124.07	164.23	+33.30	16.86
	2	6,000	197.53	42.31	113.36	155.67	+41.86	21.19
	3	9,000	296.30	57.34	191.67	249.01	+47.29	15.96
	4	6,000	197.53	38.56	133.06	171.62	+25.91	13.12
	5	6,000	197.53	42.87	122.82	165.69	+31.84	16.12
	6	6,000	197.53	37.97	123.31	161.28	+36.25	18.35
Average		6,500.0	213.99	43.20	134.72	177.92	+36.07	[a] 16.86

[a] Average nitrogen balance as a percentage of the average nitrogen consumed.

TABLE 9.—*Nitrogen balance of 6 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa during 10-day periods*—Continued

THIRD CUTTING

Part of plant and trial no.	Lamb no.	Feed eaten	Nitrogen consumed	Nitrogen voided in—			Nitrogen balance	Intake stored
				Feces	Urine	Total outgo		
Stems:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
1001	1	4,194	49.07	14.57	26.40	40.97	+8.10	16.51
	2	5,100	59.72	25.08	29.17	54.25	+5.47	9.16
	3	5,100	59.72	28.34	28.67	57.01	+2.71	4.54
	4	5,100	59.72	43.03	20.64	63.67	−3.95	
	5	5,100	59.72	30.32	29.54	59.86	−.14	
	6	5,100	59.72	28.27	35.22	63.49	−3.77	
Average		4,949.0	57.94	28.27	28.27	56.54	+1.40	[a] 2.42
Whole hay:								
1002	1	6,900	158.04	43.43	74.31	117.74	+40.30	25.50
	2	7,780	178.19	48.67	91.22	139.89	+38.30	21.49
	3	7,990	183.00	47.98	91.17	139.15	+43.85	23.96
	4	8,010	183.46	47.49	97.04	144.53	+38.93	21.22
	5	8,160	186.90	48.15	88.00	136.15	+50.75	27.15
	6	8,220	188.27	44.72	92.76	137.48	+50.79	26.98
Average		7,843.3	179.64	46.74	89.08	135.82	+43.82	[a] 24.39
Leaves:								
1003	1	5,075	148.19	35.21	92.64	127.85	+20.34	13.73
	2	7,565	220.90	49.44	132.95	182.39	+38.51	17.43
	3	8,070	235.64	50.91	137.21	188.12	+47.52	20.17
	4	8,070	235.64	52.41	149.79	202.20	+33.44	14.19
	5	8,070	235.64	56.24	132.85	189.09	+46.45	19.71
	6	8,070	235.64	48.55	144.93	193.48	+42.16	17.89
Average		7,486.7	218.61	48.79	131.73	180.52	+38.09	[a] 17.42

[a] Average nitrogen balance as a percentage of the average nitrogen consumed.

TABLE 10.—*Nitrogen-balance data, with percentages of intake computed for stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to lambs*

Part of plant and cutting	Feed eaten	Nitrogen consumed	Nitrogen voided			Nitrogen balance	Intake stored [a]
			Feces	Urine	Total outgo		
Stems:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
First	6,538	87.97	43.68	38.28	81.96	+6.01	+6.8
Second	6,229	84.28	40.87	38.90	79.77	+4.51	+5.4
Third	4,949	57.94	28.27	28.27	56.54	+1.40	+2.4
Average	5,905	76.73	37.61	35.15	72.76	+3.97	+5.2
Whole hay:							
First	5,695	106.34	36.93	81.82	118.75	−12.41	
Second	8,721	198.31	59.40	87.93	147.33	+50.98	+25.7
Third	7,843	179.64	46.74	89.08	135.82	+43.82	+24.4
Average	7,420	161.43	47.69	86.28	133.97	+27.46	+17.0
Leaves:							
First	6,192	174.46	44.82	105.09	149.37	+25.09	+14.4
Second	6,500	213.99	43.20	134.72	177.92	+36.07	+16.9
Third	7,487	218.61	48.79	131.73	180.52	+38.09	+17.4
Average	6,726	202.35	45.42	123.85	169.27	+33.08	+16.3

[a] Average nitrogen balance as a percentage of the average nitrogen consumed.

The nitrogen balances were most favorable during the feeding of second-cutting hay, all being positive. There was a marked contrast between the balances secured with the first- and second-cutting crop. The lambs ate less of the first-cutting hay, and the nitrogen intake in consequence was only 54 percent of that of the second-cutting hay rations. Four of the six lambs fed the first-cutting hay lost nitrogen. These were the only cases out of 18 in which whole hay was fed where the balances were negative. Some of the objections to short-time experiments as pointed out by Stewart (*40*) were overcome by thoroughly accustoming the lambs to the metabolism cages before proceeding with the experiments.

In the case of the stem rations of the first cutting, only 1 negative nitrogen balance was observed, whereas when stems of the second-cutting hay were fed, 3 lambs lost nitrogen. The same was true when stems of the third cutting were fed.

The various rations show negative balances for intakes of nitrogen varying from 60 to 112 g for 10-day periods. On the other hand, a positive balance was obtained with as low a nitrogen intake as 49.07 g for a 10-day period. There is no assurance that a growing lamb fed all the alfalfa hay it will consume will be kept in nitrogen equilibrium.

In the nitrogen-balance data in tables 9 and 10 the last column shows the percentage retention of feed nitrogen. The percentage retention was affected by the excess or deficiency in regard to the requirements for maintenance and growth, by the stage of maturity of the animal, and by the net protein-net energy ratio of the ration.

If the protein requirements for maintenance and growth are met as well as the smaller protein requirement for fattening and wool growth, no further storage of protein takes place, and the excess nitrogen is excreted.

BIOLOGICAL VALUE OF PROTEINS IN LEAVES AND STEMS

Differences in digestibility (*38*) do not affect the biological values of proteins, and biological values are expected to vary with the use to which the protein is put by the body. The amino-acid make-up of the protein as compared with the several separate requirements for amino acids by the animal account for differences in biological values. For any set of conditions the biological value of a protein is dependent upon its amino-acid make-up. Mitchell (*23*) pointed out that a lowering in the biological value occurred as the concentration of the protein in the ration increased, irrespective of the quantitative intake. This was true of the stem, whole-hay, and leaf rations studied. Failure to equalize the fiber content of the ration fed is not serious. An underestimation of metabolic nitrogen in a period of protein feeding leads to an overestimation of both the food nitrogen retained and absorbed. Since one is divided by the other in computing the biological value, the error is not considered serious. This fact was brought out in connection with the feeding of alfalfa stems, whole hay, and leaves, which varied in their content of crude fiber.

The data on which the computation of the biological values is based are contained in tables 11 and 12. The order of stems, whole hay, and leaves was followed throughout. The same methods of computation were followed as previously described (*26*).

TABLE 11.—*Biological values of proteins in stem, leaves, and whole hay of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods*

FIRST CUTTING

Ration and trial no.	Lamb no.[a]	Body weights			Dry matter feed intake	Nitrogen intake	Fecal nitrogen	Nitrogen in urine	Biological value of protein	Average value
		Initial	Final	Average						
		Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*		
Near-nitrogen free:										
100	100e	24.49	22.68	23.5	1,943.00	2.35	15.24	15.92		
	101w	27.22	23.81	25.5	3,290.00	3.98	21.73	8.70		
	102e	26.76	24.49	25.6	2,823.00	3.42	18.31	5.55		
101	103e	25.40	22.68	24.0	3,618.00	4.38	21.15	11.42		
	104e	24.04	21.55	22.8	2,196.00	2.66	18.27	14.22		
	105e	21.55	18.14	19.8	2,470.00	2.99	17.82	15.84		
Stems:										
102	100	19.51	19.73	19.6	5,223.08	79.73	36.07	37.55		
	101	22.91	21.77	22.3	5,752.00	87.80	42.10	51.83	48	
	102	21.71	21.32	21.5	5,752.00	87.80	42.29	29.47	71	
103	103	25.40	24.27	24.8	5,951.00	90.83	40.25	50.27	54	67. ±4.55
	104	23.59	23.59	23.6	5,951.00	90.83	54.91	28.99	81	
	105	21.09	21.55	21.3	5,951.00	90.83	46.42	31.58	80	
Whole hay:										
104	100	21.55	21.55	21.6	5,269.00	112.03	37.25	145.21		
	101	23.13	21.32	22.2	5,269.00	112.03	34.43	80.80	35	
	102	22.91	22.23	22.6	5,269.00	112.03	40.29	65.96	42	
105	103	27.22	22.68	25.0	4,733.00	100.64	35.96	72.65	33	41. ±2.27
	104	25.17	22.23	23.7	4,733.00	100.64	37.01	59.01	52	
	105	22.23	19.50	20.9	4,733.00	100.64	36.62	67.26	43	
Leaves:										
106	100	22.23	21.98	22.1	5,194.00	169.06	37.66	102.59	42	
	101	22.00	21.75	21.9	5,194.00	169.06	40.66	108.90	38	
	102	24.04	23.03	23.5	5,194.00	169.06	52.66	103.91	34	
107	103	26.31	25.86	26.1	5,540.00	180.33	41.61	110.59	41	41. ±1.41
	104	24.95	26.31	25.6	5,497.00	178.92	45.33	98.43	49	
	105	23.81	23.13	23.5	5,540.00	180.33	47.74	106.11	44	
Near-nitrogen free:										
108	100	23.81	21.55	22.7	3,221.00	3.90	14.93	8.89		
	101	24.04	22.23	23.1	2,855.00	3.45	13.80	14.95		
	102	24.49	21.09	22.8	2,467.00	2.99	10.71	11.05		
109	103	28.12	24.49	26.3	3,610.00	4.37	21.50	9.58		
	104	26.76	23.36	25.1	2,894.00	3.50	16.70	11.20		
	105	24.49	22.00	23.2	2,412.00	2.92	22.18	8.01		

SECOND CUTTING

Nitrogen free:										
500	201w	22.70	19.50	21.1	1,369.00	1.66	12.94	9.83		
	202w	20.40	17.50	19.0	1,451.27	1.76	12.62	7.26		
	[b]207w									
	204w	21.80	20.00	20.9	2,505.06	3.03	17.21	6.41		
	205w	23.60	21.50	22.6	2,419.09	2.93	15.70	8.50		
	206w	20.20	18.80	19.5	2,481.95	3.00	17.12	6.20		
Stems:										
501	201	24.70	26.80	25.7	6,681.91	98.43	43.33	36.34	74	
	202	18.60	18.40	18.5	4,684.23	69.00	31.60	42.54	48	
	207	41.30	44.90	43.1	7,852.97	115.68	49.57	44.16	80	61. ±3.37
	204	20.40	23.10	21.8	5,143.47	75.77	32.88	44.36	51	
	205	21.80	21.30	21.5	4,982.73	73.40	43.58	28.15	65	
	206	21.30	21.10	21.2	4,982.73	73.40	44.28	37.84	50	
Whole hay:										
502	201	25.90	27.70	26.8	8,193.60	204.66	57.38	89.08	61	
	202	18.40	20.90	19.7	7,488.04	187.04	55.58	62.63	70	
	207	42.20	43.10	42.6	9,490.92	237.06	65.75	130.57	52	59. ±1.69
	204	21.80	23.60	22.7	7,488.04	187.04	55.19	81.93	58	
	205	22.70	24.90	23.8	7,488.04	187.04	49.74	81.46	56	
	206	22.20	22.70	22.5	7,488.04	187.04	62.78	81.88	57	
Leaves:										
503	201	25.60	23.50	24.6	5,551.50	197.53	40.16	124.07	41	
	202	21.10	20.00	20.6	5,551.50	197.53	42.31	113.36	46	
	207	40.60	39.50	40.1	8,327.25	296.30	57.34	191.67	42	41. ± .84
	204	21.50	21.80	21.7	5,551.50	197.53	38.56	133.06	37	
	205	22.20	23.60	22.9	5,551.50	197.53	42.87	122.82	39	
	206	20.90	21.90	21.4	5,551.50	197.53	37.97	123.31	42	

[a] e=ewe and w=wether.
[b] No trial conducted.

TABLE 11.—*Biological values of proteins in stem, leaves, and whole hay of the first, second, and third cuttings of alfalfa when fed to 6 lambs as their sole ration during 10-day periods*—Continued

THIRD CUTTING

Ration and trial no.	Lamb no.	Body weights: Initial	Body weights: Final	Body weights: Average	Dry matter feed intake	Nitrogen intake	Fecal nitrogen	Nitrogen in urine	Biological value of protein	Average value
		Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*		
Nitrogen free:										
504	201	23.80	24.30	24.0	5,031.32	6.09	20.81	7.05	.....	.....
	202	22.20	20.00	21.1	3,258.94	3.94	16.98	6.99	.....	.....
	207	43.50	37.20	40.4	5,441.28	6.58	31.83	20.05	.....	.....
	204	21.80	20.60	21.2	3,340.24	4.04	16.08	9.88	.....	.....
	205	23.10	22.70	22.9	3,305.26	4.00	16.46	5.14	.....	.....
	206	21.30	18.80	20.1	3,288.25	3.98	17.96	8.24	.....	.....
Nitrogen free:										
1000	1w	23.81	20.86	22.3	2,194.01	2.65	14.42	7.54	.....	.....
	2w	24.72	23.81	24.3	2,291.38	2.77	15.17	8.36	.....	.....
	3e	28.35	26.99	27.7	3,043.38	3.68	15.74	6.82	.....	.....
	4w	29.03	26.99	28.0	2,584.40	3.13	22.95	7.27	.....	.....
	5w	31.52	29.26	30.4	3,295.11	3.99	21.00	7.30	.....	.....
	6w	33.79	29.94	31.9	3,004.82	3.64	20.37	15.79	.....	.....
Stems:										
1001	1w	22.00	21.77	21.9	3,621.24	49.07	14.57	26.40	62	65. ±1.58
	2w	25.40	24.49	24.9	4,403.51	59.72	25.08	29.17	66	
	3e	28.35	28.35	28.4	4,403.51	59.72	28.34	28.67	61	
	4w	27.67	27.22	27.4	4,403.51	59.72	43.03	20.64	76	
	5w	31.07	29.94	30.5	4,403.51	59.72	30.32	29.54	61	
	6w	30.84	29.49	30.2	4,403.51	59.72	28.27	35.22	64	
Whole hay:										
1002	1w	20.87	22.68	21.8	5,618.64	158.04	43.43	74.31	55	54. ± .62
	2w	24.04	26.54	25.3	6,335.22	178.19	48.67	91.22	53	
	3e	26.99	29.48	28.2	6,506.23	183.00	47.98	91.17	51	
	4w	26.31	29.03	27.7	6,522.51	183.46	47.49	97.04	52	
	5w	29.48	31.98	30.7	6,644.66	186.90	48.15	88.00	55	
	6w	30.16	32.21	31.2	6,693.51	188.27	44.72	92.76	57	
Leaves:										
1003	1w	23.59	23.13	23.4	4,311.19	148.19	35.21	92.64	39	42. ± .59
	2w	26.76	26.87	26.8	6,426.44	220.90	49.44	132.95	43	
	2e	30.16	30.39	30.3	6,855.43	235.64	50.91	137.21	42	
	4w	30.39	29.48	29.9	6,855.43	235.64	52.41	149.79	39	
	5w	34.02	33.34	33.7	6,855.43	235.64	56.24	132.85	44	
	6w	34.70	34.02	34.4	6,855.43	235.64	48.55	144.93	42	
Nitrogen free:										
1004	1w	24.04	23.13	23.6	3,042.30	3.68	16.25	8.56	.....	.....
	2w	27.22	24.49	25.9	2,042.20	2.47	14.53	10.19	.....	.....
	3e	33.79	31.52	32.7	3,882.50	4.70	21.78	9.47	.....	.....
	4w	30.39	27.67	29.0	2,804.30	3.39	17.32	10.88	.....	.....
	5w	32.66	30.84	31.8	3,334.80	4.04	18.78	8.57	.....	.....
	6w	37.19	34.02	35.6	4,095.10	4.96	21.21	12.54	.....	.....

TABLE 12.—*Biological values of proteins of stems, whole hay, and leaves of the first second, and third cuttings of alfalfa when fed to lambs*

Part of plant	First cutting: Number of trials	First cutting: Biological value of proteins	Second cutting: Number of trials	Second cutting: Biological value of proteins	Third cutting: Number of trials	Third cutting: Biological value of proteins	Weighted averages of all biological values
Stems	5	67±4.55	6	61±3.77	6	65±1.58	64±1.93
Whole hay	6	41±2.27	6	59±1.69	6	54±0.62	51±1.55
Leaves	6	41±1.41	6	41±0.84	6	42±0.59	41±0.56

The level of protein intake for the stems, whole-hay, and leaf rations was approximately 8, 13, and 18.5 percent, while the fiber percentage in the same order was 40, 28, and 16. These differences in the chemical make-up of the rations should be kept in mind in comparing the biological values. Comparisons limited to the values for stems, leaves, or whole hay therefore appear to carry more weight than do those in which figures for stems are directly compared with those for leaves or the whole hay.

The somewhat lower biological values obtained for the stems of the second cutting, as compared with those for the first and the third were hardly in line with the biological value for the proteins of whole hay of the second cutting which was higher than that secured with the proteins of the whole hay of the first or third cuttings.

As pointed out (*9*, *10*, *14*, *43*), the biological values of alfalfa proteins are relatively low, approximating those obtained by other workers with proteins of the corn grain.

CALCIUM AND PHOSPHORUS BALANCE STUDIES

Fifty-four complete calcium and the same number of phosphorus balances show further differences in the three cuttings (tables 13 and 14).

TABLE 13.—*Calcium and phosphorus balances of 18 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa as their sole ration during 10-day periods*

FIRST CUTTING

Part of plant and trial no.	Lamb no.	Calcium consumed	Calcium voided			Calcium balance	Phosphorus consumed	Phosphorus voided			Phosphorus balance
			Feces	Urine	Total			Feces	Urine	Total	
Stems:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
102	1	43.82	52.15	0.10	52.25	−8.43	6.16	6.79	0.04	6.83	−0.67
	2	48.26	52.94	.16	53.10	−4.84	6.78	9.22	.05	9.27	−2.49
	3	48.26	60.79	.14	60.93	−12.67	6.78	6.41	.03	6.44	+.34
103	4	49.92	47.19	.08	47.27	+2.65	7.01	7.86	.04	7.90	−.89
	5	49.92	61.72	.12	61.84	−11.92	7.01	9.86	.03	9.89	−2.88
	6	49.92	61.27	.08	61.35	−11.43	7.01	8.59	.02	8.61	−1.60
Average		48.35	56.01	.11	56.12	−7.77	6.79	8.12	.04	8.16	−1.37
Whole hay:											
104	1	84.72	91.06	1.03	92.09	−7.37	7.93	5.81	.09	5.90	+2.03
	2	84.72	82.42	.38	82.80	+1.92	7.93	6.25	.09	6.34	+1.59
	3	84.72	83.77	.13	83.90	+.82	7.93	6.77	.04	6.81	+1.12
105	4	76.11	76.21	.31	76.52	−.41	7.12	7.96	.08	8.04	−.92
	5	76.11	105.74	.14	105.88	−29.77	7.12	8.70	.05	8.75	−1.63
	6	76.11	79.81	.21	80.02	−3.91	7.12	7.33	.05	7.38	−.26
Average		80.42	86.50	.37	86.87	−6.45	7.52	7.14	.07	7.21	+.31
Leaves:											
106	1	170.44	153.16	.59	153.75	+16.69	10.83	8.96	.12	9.08	+1.75
	2	170.44	160.19	.46	160.65	+9.79	10.83	7.79	.12	7.91	+2.92
	3	170.44	166.42	.61	167.03	+3.41	10.83	11.40	.07	11.47	−.64
107	4	181.81	153.59	.29	153.88	+27.93	11.55	7.93	.08	8.01	+3.54
	5	180.38	75.17	.20	75.37	+105.01	11.46	8.43	.05	8.48	+2.98
	6	181.81	159.00	.40	159.40	+22.41	11.55	9.20	.18	9.38	+2.17
Average		175.89	144.59	.43	145.02	+30.87	11.18	8.95	.10	9.06	+2.12

TABLE 13.—*Calcium and phosphorus balances of 18 lambs when fed stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa as their sole ration during 10-day periods*—Continued

SECOND CUTTING

Part of plant and trial no.	Lamb no.	Calcium consumed	Calcium voided			Calcium balance	Phosphorus consumed	Phosphorus voided			Phosphorus balance
			Feces	Urine	Total			Feces	Urine	Total	
Stems:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
501	1	57.93	41.20	0.14	41.34	+16.59	13.95	9.36	0.21	9.57	+4.38
	2	40.61	34.42	.08	34.50	+6.11	9.78	9.05	.10	9.15	+.63
	3	68.09	53.19	.05	53.24	+14.85	16.39	10.93	.18	11.11	+5.28
	4	44.59	31.43	.05	31.48	+13.11	10.74	7.04	.16	7.20	+3.54
	5	43.20	32.84	.02	32.86	+10.34	10.40	7.20	.09	7.29	+3.11
	6	43.20	28.55	.02	28.57	+14.63	10.40	6.12	.09	6.21	+4.19
Average		49.60	36.94	.06	37.00	12.60	11.94	8.28	.14	8.42	+3.52
Whole hay:											
502	1	135.86	111.18	.37	111.55	+24.31	20.82	10.72	.21	10.93	+9.89
	2	124.17	108.27	.19	108.46	+15.71	19.02	11.62	.07	11.69	+7.33
	3	157.38	133.08	.23	133.31	+24.07	24.11	15.91	.25	16.16	+7.95
	4	124.17	109.14	.33	109.47	+14.70	19.02	12.74	.22	12.96	+6.06
	5	124.17	113.49	.08	113.57	+10.60	19.02	13.37	.16	13.53	+5.49
	6	124.17	112.96	.36	113.32	+10.85	19.02	13.12	.15	13.27	+5.75
Average		131.65	114.69	.26	114.95	+16.70	20.17	12.91	.18	13.09	+7.08
Leaves:											
503	1	141.28	112.00	.21	112.21	+29.07	17.34	3.60	1.48	5.08	+12.26
	2	141.28	120.97	.11	121.08	+20.20	17.34	3.25	.76	4.01	+13.33
	3	211.92	167.72	.15	167.87	+44.05	26.01	2.39	1.44	3.83	+22.18
	4	141.28	120.95	1.05	122.00	+19.28	17.34	.77	1.21	1.98	+15.36
	5	141.28	123.55	.07	123.62	+17.66	17.34	2.46	.78	3.24	+14.10
	6	141.28	116.78	.24	117.02	+24.26	17.34	2.13	.54	2.67	+14.67
Average		153.05	127.00	.30	127.30	+25.75	18.78	2.43	1.04	3.47	+15.31

THIRD CUTTING

Stems:											
1001	1	41.94	35.56	0.15	35.71	+6.23	3.87	3.93	0.10	4.03	−0.16
	2	51.00	51.76	.13	51.89	−.89	4.70	5.57	.13	5.70	−1.00
	3	51.00	55.79	.06	55.85	−4.85	4.70	5.14	.11	5.25	−.55
	4	51.00	52.68	.03	52.71	−1.71	4.70	4.74	.09	4.83	−.13
	5	51.00	55.64	.08	55.72	−4.72	4.70	4.98	.16	5.14	−.44
	6	51.00	50.75	.05	50.80	+.20	4.70	6.17	.15	6.32	−1.62
Average		49.49	50.36	.08	50.45	−.96	4.56	5.09	.12	5.21	−.65
Whole hay:											
1002	1	161.12	132.60	.30	132.90	+28.22	10.31	6.32	.10	6.42	+3.89
	2	181.66	151.75	.40	152.15	+29.51	11.67	7.71	.13	7.84	+3.83
	3	186.57	152.82	.22	153.04	+33.53	11.98	7.78	.13	7.91	+4.07
	4	187.03	157.93	.22	158.15	+28.88	12.02	6.59	.13	6.72	+5.30
	5	190.54	157.97	.36	158.33	+32.21	12.24	7.69	.16	7.85	+4.39
	6	191.94	153.55	.70	154.25	+37.69	12.33	7.36	.16	7.52	+4.81
Average		183.14	151.10	.37	151.47	+31.67	11.76	7.24	.14	7.38	+4.38
Leaves:											
1003	1	146.24	138.11	3.18	141.29	+4.95	9.12	5.56	.85	6.41	+2.71
	2	217.99	191.62	3.29	194.91	+23.08	13.60	7.58	1.13	8.71	+4.89
	3	232.54	202.90	4.12	207.02	+25.52	14.51	7.70	.84	8.54	+5.97
	4	232.54	215.22	7.56	222.78	+9.76	14.51	9.45	1.58	11.03	+3.48
	5	232.54	218.82	4.21	223.03	+9.51	14.51	8.52	1.34	9.86	+4.65
	6	232.54	202.04	7.16	209.20	+23.34	14.51	7.44	1.44	8.88	+5.63
Average		215.73	194.78	4.92	199.70	+16.03	13.46	7.71	1.20	8.91	+4.55

TABLE 14.—*Calcium and phosphorus balance data computed for stems, whole hay, and leaves of the first, second, and third cuttings of alfalfa when fed to lambs*

Cutting	Calcium consumed	Calcium voided			Calcium balance	Phosphorus consumed	Phosphorus voided			Phosphorus balance
		Feces	Urine	Total			Feces	Urine	Total	
Stems:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
First	48.35	56.01	0.11	56.12	−7.77	6.79	8.12	0.04	8.16	−1.37
Second	49.60	36.94	.06	37.00	+12.60	11.94	8.28	.14	8.42	+3.52
Third	49.49	50.36	.08	50.45	−.96	4.56	5.09	.12	5.21	−.65
Average	49.15	47.77	.08	47.86	+1.29	7.76	7.16	.10	7.26	+.50
Whole hay:										
First	80.42	86.50	.37	86.87	−6.45	7.52	7.14	.07	7.21	+.31
Second	131.65	114.69	.26	114.95	+16.70	20.17	12.91	.18	13.09	+7.08
Third	183.14	151.10	.37	151.47	+31.67	11.76	7.24	.14	7.38	+4.38
Average	131.74	117.43	.33	117.76	+13.98	13.15	9.10	.13	9.23	+3.92
Leaves:										
First	175.89	144.59	.43	145.02	+30.87	11.18	8.95	.10	9.06	+2.12
Second	153.05	127.00	.30	127.30	+25.75	18.78	2.43	1.04	3.47	+15.31
Third	215.73	194.78	4.92	199.70	+16.03	13.46	7.71	1.20	8.91	+4.55
Average	181.56	155.46	1.88	157.34	+24.22	14.47	6.36	.78	7.15	+7.32

The stems of the second cutting were better sources of calcium and phosphorus than those of the first and third cuttings. The intake of calcium per lamb for the 10-day periods varied from 40.6 to 68.1 g.

The phosphorus balances for the second-cutting stem ration were all positive. The 10-day intake of phosphorus per lamb varied from 9.8 to 16.4 g, and its storage from 0.6 to 5.3 g.

In contrast with these figures are those for the stem ration of first-cutting hay. The calcium intake per lamb was more or less uniform, ranging from 43.8 to 49.9 g per lamb for the 10-day period. However, every lamb except no. 4 lost from 4.8 to 12.7 g of calcium. One lamb gained 2.7 g. The lambs consumed from 6.2 to 7 g. per head of phosphorus in the 10-days, and every lamb but one showed losses. These losses varied from 0.7 to 2.9 g., while a gain of only 0.3 g. is shown for lamb 3.

Results with stems of the third cutting showed 2 positive and 4 negative calcium balances, and all negative phosphorus balances. The stems, because of their bulk and low percentage of both calcium and phosphorus, when fed as the sole feed, resulted in negative balances in most cases, the ration of stems of the second-cutting hay being an exception.

Whole-hay rations showed the second and third cuttings to be good sources of both calcium and phosphorus, as evidenced by strongly positive balances. In contrast were the results when lambs were fed first-cutting hay. The calcium and phosphorus intake was lower than for the second and third cuttings, and all the lanbs but two showed losses of calcium.

The leaf rations supplied most calcium. They were also highest in phosphorus in the first and third cuttings. The second-cutting leaf rations on the average, however, were lower in phosphorus. All the leaf rations produced positive calcium balances, and the data show that the leaves are excellent sources of calcium.

The leaf rations were also apparently good sources of phosphorus. When the first-cutting leaves were fed, all but one lamb stored some

phosphorus, the quantities varying from 1.8 to 3.5 g. per lamb for the 10-day period. One lamb lost 0.6 g. of phosphorus. The leaves of the second cutting excelled all others as sources of phosphorus, and third-cutting leaves ranked second in this respect.

The average results in table 14 show that a lamb fed alfalfa stems for 10 days will consume approximately 49 g of calcium, of which slightly less than 1.3 g will be retained by the body. These results, however, may be misleading, because losses occurred when first- and third-cutting stems were fed, and the excellent storage of calcium when second-cutting stems were fed more than offset the losses. The same was true of phosphorus. The average intake of phosphorus per lamb for the 10-day period was approximately 7.8 g, 0.5 g being stored. Again the second-cutting stems resulted in excellent storage of phosphorus, so as to offset the negative results secured with rations of first- and third-cutting stems when the balances were averaged.

An average of all data for three cuttings of whole hay shows that a lamb consumed 131.7 g of calcium, of which 14 g were retained. When first-cutting hay was fed, losses resulted. The feeding of third-cutting hay resulted in the most favorable storage of calcium. The calcium retention of 14 g per lamb as an average for all lambs was accompanied by a storage of 5 g of phosphorus. Actual losses of calcium occurred when first-cutting hay was fed. The second cutting was the best source of phosphorus.

The results as summarized for leaf rations show an intake of 181.6 g of calcium for the 10-day period, accompanied by a storage of 24 g. All balances were positive, and strangely enough the feeding of leaves of first-cutting hay resulted in the most favorable storage of calcium.

The average intake of phosphorus in the leaf rations for this period was 14.5 g, of which 7 g was stored. Second-cutting leaves produced the most favorable balances.

Table 14 shows strikingly the value of leaves as sources of available calcium and phosphorus. The whole hay ranked second, but the average results with first-cutting whole hay showed actual losses of calcium and phosphorus.

The results with stems show negative balances for all but the second-cutting stems. Throughout the entire study the results with second-cutting stems and leaves show them especially to be excellent sources of calcium and phosphorus. Most of the phosphorus was excreted by way of the intestines, as was shown by Ross et al. (*31*).

SUMMARY AND CONCULSIONS

In 10 experiments reported in this paper, 67 to 83 percent of the total protein of the alfalfa plant was contained in the leaves, as well as 71 to 85 percent of the calcium, and 46 to 79 percent of the phosphorus.

The leaves exerted a marked diuretic effect on lambs in the decreasing order of first, second and third cuttings.

The trend was toward an almost equal partition of excretory nitrogen between feces and urine when stems were fed; with the exception of the second cutting, approximately 67 percent of the nitrogen was found in the urine with whole-hay rations; 70 to 78 percent of the nitrogen excreted when leaf rations were fed was found in the urine.

The coefficients of apparent digestibility, averages for 18 lambs and of 3 cuttings were as follows: (1) For the stems, dry matter 46.6, crude protein 51.1, crude fiber 39.0, fat 47.9, and nitrogen-free extract 58.9; (2) for the whole hay, dry matter 56.3, crude protein 69.7, crude fiber 40.0, fat 24.0, and nitrogen-free extract 71.0; (3) for the leaves, dry matter 66.3, crude protein 77.4, crude fiber 55.5, fat 29.7, and nitrogen-free extract 75.9. Coefficients for the stems, whole hay, and leaves of each of the three cuttings are reported separately.

A given weight of leaves was almost 3.57 times as efficient as the same weight of stems in supplying digestible protein, and the leaves were less bulky.

The percentage of digestible nutrients in the stems, whole hay, and leaves of alfalfa, average for three cuttings and with 18 lambs were as follows: Stems, 4.17 percent digestible crude protein and 41.55 percent total digestible nutrients; whole hay, 9.77 percent digestible crude protein and 48.43 percent total digestible nutrients; leaves, 14.87 percent digestible crude protein, and 57.82 percent total digestible nutrients.

When alfalfa stems (average of all three cuttings) were fed, 5.2 percent of the nitrogen was stored; when whole hay was fed, 17 percent was retained; when leaves were fed, 16.3 percent was stored. The slightly lower retention when leaves were fed was possibly due to an excessive supply of nitrogen above the optimum necessary for maintenance and growth and for the small requirements incident to slight fattening and wool growth.

The weighted averages of biological values of the proteins from the three cuttings as determined with 18 growing lambs were as follows: (1) Stems, 64; (2) whole hay, 51; (3) leaves, 41. The variation in values are attributed to the differences in protein concentration of the stem, leaf, and whole-hay rations.

Lambs fed stems of the three cuttings consumed during a 10-day period a total of 49.15 g of calcium and 7.76 g of phosphorus. The balances show a retention of 1.29 g of calcium and 0.50 g of phosphorus.

When whole alfalfa hay was fed, the lambs consumed on the average 131.74 g of calcium each 10 days, and of this they retained 13.98 g. The phosphorus intake was 13.15 g and the retention 3.92 g.

The leaf rations (all three cuttings) contained 181.56 g of calcium per lamb for the 10-day period of which 24.22 g were retained. The phosphorus content was 14.47 g and the retention 7.32 g.

Complete data for each lamb fed stems, leaves, and whole hay of the three cuttings are included. They support the conclusion that alfalfa leaves are important sources of calcium and phosphorus, in spite of the unfavorable ratio in which these are found.

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EFFECT OF THE RIPENING PROCESS OF CHEESE ON THE NUTRITIVE VALUE OF THE PROTEIN OF MILK CURD [1]

By JESSIE R. BEADLES, *assistant in animal nutrition*, J. H. QUISENBERRY, *assistant in animal genetics*, F. I. NAKAMURA, *assistant in animal nutrition*, and H. H. MITCHELL, *professor and chief in animal nutrition, Illinois Agricultural Experiment Station*

INTRODUCTION

The ripening of cheese is essentially a hydrolysis of the paracasein in the rennet curd through the agency of proteases (*5*).[2] During this proteolysis the concentration of water-soluble nitrogen, as also of nonprotein and amino nitrogen, in the cheese increases rather evenly with time (*6*). Insofar as these changes predominate in the ripening of cheese, there is no reason to suspect that the nutritive value of the rennet-curd protein would be affected. However, the products of this proteolysis, perhaps largely through the influence of bacteria, undergo certain secondary reactions, involving oxidation, reduction, deamination, and decarboxylation (*2*, *15*). The products of these reactions may differ widely from the products of simple proteolysis with respect to their value in animal nutrition, though their occurrence in cheeses ripened mainly by other agencies than bacteria may be of minor importance. Nelson (*10*) reported the presence of indole and phenols in Limburger cheese, while Camembert cheese contained a trace of indole but no phenols.

No attempt appears to have been made to determine whether and to what extent the nutritive value of the protein of the rennet curd has been affected by the chemical changes incident to ripening. The present and the increasing importance of cheese in the American diet warrants a thorough study of this subject. This paper represents a contribution to it, involving a series of controlled-feeding experiments and metabolism studies on rats.

MATERIAL STUDIED

The cheeses studied were American Cheddar, Roquefort, Swiss, and Limburger. The latter three varieties were furnished by the Kraft-Phenix Cheese Corporation of Chicago. The American Cheddar cheese, and the fresh rennet curd from cow's milk, with which all cheeses were compared, were supplied by the Department of Dairy Husbandry of the University of Illinois.

The fresh cheese was ground, mixed, and sampled for chemical analysis. It was then dried at a low temperature before a blower, ground, and extracted with ether to remove practically all the fat. The dried residue was then analyzed for nitrogen and fat. The ether-soluble material removed from each cheese was also analyzed for nitrogen.

The chemical composition of the fresh cheese is given in table 1. The percentage of nitrogen varied from 9.75 (Roquefort) to 13.25

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[2] Reference is made by number (italic) to Literature Cited, p. 18.

(milk curd) in the dried and extracted cheese and in the dried milk curd used in the preparation of the experimental rations. The fat content of these preparations was low (about 0.2 percent). The nitrogen content of the ether-soluble material removed from the cheese was very small, ranging from 0.0037 to 0.031 percent. It is evident that only an insignificant portion of the cheese nitrogen was removed from the experimental samples by ether extraction.

TABLE 1.—*Chemical composition of the fresh cheese*

Variety	Water	Protein (N×6.38)	Fat	Ash	Calcium	Phosphorus	Gross energy per gram
American Cheddar:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*
Sample 1	35.33	25.26	28.30	4.04	0.845	0.534	4.46
Sample 2	31.81	24.56	31.17	3.77			4.90
Swiss	35.70	32.03	17.63	4.05	1.05	.728	4.25
Roquefort:							
Sample 1	36.84	22.14	33.35	7.38			
Sample 2	40.33	20.03	32.08	6.89	.550	.424	4.06
Limburger:							
Sample 1	48.00	21.25	26.37	3.68	.656	.418	3.83
Sample 2	57.46	21.56	28.65	3.59	.524	.368	4.17

In a sample of Cheddar cheese and a sample of Limburger cheese the writers attempted, by the use of Richmond's method (*13, p. 231*), to differentiate the primary products of ripening (precipitated as basic copper compounds) and the secondary products of ripening (not so precipitated) in the water-soluble constituents of the cheese. In Cheddar cheese it was found that 29 percent of the total nitrogen was water soluble, and of the water-soluble nitrogen, 62.6 percent was not precipitated by copper sulphate ($CuSO_4$) and sodium hydroxide (NaOH). In Limburger cheese, these percentages were, respectively, 34.6 and 56.6.

EXPERIMENTAL PROCEDURE

The first comparisons of cheese protein and milk-curd protein were made by the paired-feeding method, rats being used as subjects. Eight or nine pairs of rats were used in each comparison. Pair mates were always of the same sex, approximately of the same initial weight, and in the large majority of cases they were litter mates. The rats were confined in individual wire cages of the usual design, and the food consumption of pair mates was equalized, so that the only difference in their treatment was the difference in composition of the rations compared. In each comparison of cheese with milk curd, one rat in each pair received the cheese ration and the other the milk-curd ration. These rations contained the same nonprotein ingredients and practically the same percentage of nitrogen, and they had very nearly the same gross energy value as determined by the bomb calorimeter. The nitrogen content of all rations was brought close to 1.28 percent, equivalent to 8 percent of crude protein. This level of protein will permit considerable growth if the protein possesses a high biological value and yet will not promote maximum growth with the best of proteins, a point of great importance in the technic of protein comparison.

All the experimental rations contained 4 or 4.5 percent, either of the original Osborne-Mendel salt mixture (*12*) or, later, of the modified

mixture proposed by Wesson (*14*), 8 percent of clarified butterfat, 2 percent of cod-liver oil, 10 percent of sucrose, 1 percent of sodium chloride, and 1 percent of dried yeast. Each ration also contained enough of the dried extracted cheese or the dried rennet curd (approximately 10 percent) to provide 1.28 percent of nitrogen, and enough starch to make up 100 percent. The rations were apparently all deficient in vitamin B_1, since shortly after the start of each experiment it was necessary to give a few drops of tiki-tiki extract to each rat to stimulate a failing appetite. In this, as in every other respect, pair mates were treated exactly alike.

The rats were weighed for 3 consecutive days at the beginning and the end of the feeding period, the average of each group of three weights constituting the initial or the final weight of the rat. Weekly weights were also taken throughout the experiments which continued for 7 or 8 weeks.

In the process of equating the food intakes of pair mates, the amount of food offered to the rats in each pair was increased as long as both rats cleaned up their dishes and was reduced whenever food residues remained in either of the dishes at the end of the day. Notes were kept of the number of times that each rat refused food throughout the experiment. A comparison of these food refusals between pair mates affords information of the comparative avidity with which the experimental rations were being consumed, or possibly of their comparative palatability. Such information is of interest in testing the frequently expressed (or implied) opinion that the better balanced of any two rations will always be consumed in the greater amount in a feeding experiment in which unrestricted access to the feed is permitted.

At the end of each experiment, the rats used therein were killed with ether, and their body lengths from mouth to anus were measured. Differences in the stage of growth between pair mates would presumably be the only gross factor determining differences in body length, while other gross factors, such as differences in the deposition of fat, would affect body weight.

EXPERIMENTAL RESULTS

GROWTH-PROMOTING VALUE OF THE PROTEIN OF CHEESE AND OF RENNET MILK CURD

The results of these paired-feeding tests are summarized in tables 2, 3, 4, and 5, while a statistical summary is given in table 6. In all tests but that of the Limburger cheese the results favored the cheese as a source of protein over the milk curd. However, in only one comparison the Swiss cheese with milk curd are the differences between pair mates significant. In this case the rat receiving the cheese ration gained faster than its pair mate in 7 of the 8 pairs, while of the weekly comparisons of gains 39.5 favored the cheese ration and only 16.5 favored the milk-curd ration. When the total gains are analyzed by Student's method (1) the mean difference in gain between pair mates is 7.25 grams (favoring the cheese ration), the standard deviation of differences is 7.15 grams, and the ratio z is 1.01. For $n=8$ and $z=1.01$ the probability that the mean difference was the result of a fortuitous combination of factors common to both groups of rats is only 0.016, and is thus so small (equivalent to 1 of 62 events) that it may be neglected. It may

be concluded, therefore, that the differences observed between pair mates in rate of gain in weight was produced by the difference in the character of the dietary proteins. However, no consistent differences between pair mates were noted with respect to body length (4 comparisons favoring one ration and 4 the other), so that there may be a legitimate objection to interpreting the difference in rate of gain on the two rations as a difference in rate of growth.

The Roquefort cheese also promoted greater gains in weight than the milk curd in most of the experimental pairs. Analysis of this experiment, gives $M = +5.75$, $s = 10.35$, $z = 0.56$, and $P = 0.11$. This probability is too large to be neglected, so that the possibility that the outcome was a result of chance cannot be removed.

In all comparisons the cheese rations were considerably less frequently refused; in other words, they were the most palatable, whether or not they promoted the most rapid gains in weight.

TABLE 2.—*Comparison of the growth-promoting value of the protein of Cheddar cheese and of rennet milk curd by the paired-feeding method during a feeding period of 60 days*

Item	Pair 1, males		Pair 2, females		Pair 3, females		Pair 4, females		Pair 5, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	151	154	150	145	143	151	127	134	139	142
Initial weight.....do....	85	90	91	87	81	86	48	48	49	51
Gain.....do....	66	64	59	58	62	65	79	86	90	91
Body length.....centimeters..	19.4	19.9	19.0	18.7	18.6	18.9	18.3	18.1	18.3	18.3
Total food.....grams..	515	515	512	512	520	520	503	503	521	521

Item	Pair 6, females		Pair 7, males		Pair 8, males		Pair 9, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	111	118	118	119	121	120	115	116
Initial weight.....do....	35	37	36	37	38	39	37	37
Gain.....do....	76	81	82	82	83	81	78	79
Body length.....centimeters..	17.4	17.7	18.2	18.6	18.2	18.3	17.7	17.9
Total food.....grams..	458	458	448	448	429	429	440	440

TABLE 3.—*Comparison of the growth-promoting value of the protein of Roquefort cheese and of rennet milk curd by the paired-feeding method during a feeding period of 56 days*

Item	Pair 1, males		Pair 2, males		Pair 3, males		Pair 4, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	111	128	122	133	97	115	126	112
Initial weight.....do....	67	66	71	70	63	63	61	63
Gain.....do....	44	62	51	63	34	52	65	49
Body length.....centimeters..	17.9	18.2	18.3	18.8	17.7	17.7	17.8	17.6
Total food.....grams..	447	447	481	481	379	380	422	425

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	128	133	118	126	126	126	133	134
Initial weight.....do....	65	66	63	64	61	61	65	63
Gain.....do....	63	67	55	62	65	65	68	71
Body length.....centimeters..	18.2	18.1	18.0	18.4	18.0	17.8	17.8	18.3
Total food.....grams..	408	408	402	402	446	448	451	451

TABLE 4.—*Comparison of the growth-promoting value of the protein of Swiss cheese and of rennet milk curd by the paired-feeding method during a feeding period of 50 days*

Item	Pair 1, males		Pair 2, males		Pair 3, females		Pair 4, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	152	153	129	143	133	133	123	132
Initial weight.....do....	65	68	64	56	66	63	42	30
Total gain.....do....	87	85	65	87	67	70	81	93
Body length.....centimeters..	19.6	19.6	18.8	18.6	19.0	18.8	18.9	18.8
Total food.....grams..	477	477	423	423	430	430	421	421

Item	Pair 5, females		Pair 6, females		Pair 7, males		Pair 8, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	121	124	124	129	123	127	111	122
Initial weight.....do....	38	39	39	39	38	38	38	37
Gain.....do....	83	85	85	90	85	89	73	85
Body length.....centimeters..	17.8	18.0	18.3	18.3	18.7	18.8	17.3	17.6
Total food.....grams..	440	440	450	450	415	415	400	400

TABLE 5.—*Comparison of the growth-promoting value of the protein of Limburger cheese and of rennet milk curd by the paired-feeding method during a feeding period of 50 days*

Item	Pair 1, males		Pair 2, males		Pair 3, females		Pair 4, females	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	130	138	153	145	136	137	148	145
Initial weight.....do....	49	53	60	57	51	54	56	54
Gain.....do....	81	85	93	88	85	83	92	91
Body length.....centimeters..	19.1	19.0	19.7	19.6	18.9	18.5	19.2	18.8
Total food.....grams..	444	444	457	457	459	459	512	512

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, males	
	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese	Milk curd	Cheese
Final weight.....grams..	116	115	121	125	121	119	100	102
Initial weight.....do....	39	41	43	42	42	43	37	36
Gain.....do....	77	74	78	83	79	76	63	66
Body length.....centimeters..	18.3	18.2	18.5	18.4	17.8	18.3	17.2	17.7
Total food.....grams..	373	373	433	433	397	397	350	350

TABLE 6.—*Statistical data on the paired-feeding experiment*

Item	Milk curd	Cheddar cheese	Milk curd	Roquefort cheese	Milk curd	Swiss cheese	Milk curd	Limburger cheese
Number of food refusals	103	33	105	49	29	12	34	4
Comparison of weekly gains of pair mates [1]	33.5	38.5	25	31	16.5	39.5	29.5	26.5
Comparison of total gains of pair mates [1]	3.5	5.5	1.5	6.5	1	7	5	3
Comparison of body length of pair mates [1]	2.5	6.5	3.5	4.5	4	4	6	2

[1] The figures in these comparisons represent the number of comparisons favoring either the rat receiving milk curd or the rat receiving cheese. In the case of equal measurements (gains in weight or body lengths) each pair mate is credited with 0.5.

COMPARATIVE DIGESTIBILITY OF CHEESE PROTEIN AND MILK-CURD PROTEIN

The comparative digestibility of cheese and milk-curd protein was determined in two of the paired-feeding experiments, those involving Roquefort and Limburger cheese. The collection periods were of 7 days' duration, throughout which the intake of food was kept the same. The coefficients of apparent digestibility for pair mates receiving the same amounts of food are summarized in table 7. For all pairs of rats the milk-curd protein was digested more completely. In one series of pairs the average coefficient for milk-curd protein was 89.65 and that for Roquefort-cheese protein 87.28. In the other series of pairs the average coefficient for the milk-curd protein was 88.78 and that for Limburger-cheese protein 87.22. Furthermore, in a later test (table 9), it was found that Swiss-cheese protein was rather consistently less digestible than milk-curd protein, the average digestion coefficients being, respectively, 89.12 and 90.53.

TABLE 7.—*Coefficients of apparent digestibility of the protein of rennet milk curd and of Roquefort and Limburger cheese for the different pairs of rats*

Pair no.	Milk curd	Roquefort cheese	Milk curd	Limburger cheese	Pair no.	Milk curd	Roquefort cheese	Milk curd	Limburger cheese
1	89.2	86.3	88.4	88.2	6	88.9	86.3	89.0	86.2
2	89.7	87.8	87.4	86.8	7	89.8	86.7	87.8	86.8
3	89.0	87.3	88.6	86.9	8	89.2	88.0	90.5	89.9
4	91.2	86.3	89.6	87.2					
5	90.1	89.5	88.9	85.8	Average	89.65	87.28	88.78	87.22

It thus appears that cheese protein supports a more rapid gain in weight in albino rats than does milk-curd protein, although it is clearly, if only slightly, less digestible. This somewhat anomalous situation is explainable on three possible grounds: (1) The voluntary activity of the rats on the cheese ration was enough less than that of those on the milk-curd ration to reduce the dietary energy available for gains in weight to an extent that compensated (or more than compensated) for the less complete digestibility of the cheese protein; or (2) the biological value of cheese protein is greater than that of milk-curd protein, and enough greater partly to offset the less complete digestibility, and in one case (Swiss cheese) to more than offset it; or (3) the gains in weight produced on the cheese rations contain less protein and more fat than those produced on the milk-curd rations.

Concerning the first possible explanation, the writers have no information to offer at all. Nevertheless, it is certainly well to keep in mind that the results of feeding experiments may be modified, or even determined by a differential effect on the voluntary activity of the rats of the two rations being compared. For example, if one ration, possibly because of a poorer balance of nutrients in it, depresses voluntary muscular activity, it may promote as great a rate of gain in body weight as a better balanced ration, even when consumed in no greater amounts, because a greater proportion of it will be available for increase in weight. The effect of qualitatively different rations upon the activity of experimental animals in nutrition laboratories is practically a virgin field of study, although in the interest of the refinement of the technic of feeding experiments, it is a field that needs careful investigation.

RELATIVE BIOLOGICAL VALUES OF MILK-CURD AND CHEESE PROTEINS

To determine the relative biological values of the proteins of milk curd and of cheese, a study was made of Swiss cheese, which had given clear indications in the paired-feeding experiments of a greater capacity to promote gains in weight than had milk curd. Ten rats were used in these experiments. The plan of the experiment, as well as the technic of the metabolism work, was essentially the same as that developed in this laboratory and described elsewhere (*3*). The 10 rats were used as 2 groups of 5 each, and the plan of feeding was as follows:

Rats 1 to 5, inclusive	Rats 6 to 10, inclusive
Period 1. Standardizing ration	Standardizing ration
Period 2. Milk-curd ration	Swiss-cheese ration
Period 3. Swiss-cheese ration	Milk-curd ration
Period 4. Milk-curd ration	Swiss-cheese ration
Period 5. Standardizing ration	Standardizing ration

The experimental rations had the composition shown in table 8.

TABLE 8.—*Composition of rations used in studying the relative biological values of the proteins of milk curd and of Swiss cheese*

Constituent	Standardizing ration	Milk-curd ration	Swiss-cheese ration
	Percent	*Percent*	*Percent*
Dried, fat-free whole egg	5.47		
Dried rennet-milk curd			10.60
Dried, fat-free Swiss cheese		10.13	
Modified Osborne-Mendel salts	4.5	4.5	4.5
Clarified butterfat	8	8	8
Cod-liver oil	2	2	2
Sucrose	10	10	10
NaCl	1	1	1
Harris yeast vitamin powder	0.3	0.3	0.3
Starch	66.73	62.07	61.6
Pigment	2	2	2
Protein content	4.37	8.39	8.34

In order to improve the consumption of the experimental rations, 3 drops of tiki-tiki extract (containing 9.7 mg of nitrogen) were given to each rat daily when signs of a diminished appetite became apparent.

In these metabolism experiments feces markers were used to differentiate the feces of the experimental periods from those of the preceding and following preliminary (or transition) periods. On the first day of an experimental period the ration contained 2 percent of ferric oxide (Fe_2O_3), which colored the first day's feces a brilliant red; for the remaining 6 days of the period 2 percent of barium sulphate ($BaSO_4$) replaced the ferric oxide, giving to the feces a lighter than normal appearance; then again on the first day following the termination of the experimental period, the ration containing 2 percent of ferric oxide was given.

The purpose of the initial and final standardizing periods was to determine the excretion of body nitrogen in feces (the so-called "metabolic nitrogen") and in urine (the endogenous nitrogen), the former being dependent upon the dry matter consumed and the latter upon the body weight of the rat. The average excretion of metabolic nitrogen in the feces was 1.2 mg and 1.37 mg per gram of food consumed in the two periods. The endogenous nitrogen output

in the urine per 100 g body weight averaged 22.0 mg in the first standardizing period and 20.7 mg in the second. These factors differ somewhat in the two periods for each rat, and in computing the body's contribution to urine and feces in the intermediate experimental periods, they are assumed to change in a linear fashion from the initial to the final value determined.

TABLE 9.—*Nitrogen metabolism of rats on milk-curd and Swiss-cheese rations*

[Results expressed on the daily basis]

RATS 1 TO 5

Ration and rat no.	Initial body weight	Final body weight	Food intake	Nitrogen intake	Nitrogen in feces	Nitrogen in urine
	Grams	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*
Standardizing ration:						
1	48	59	8.1	.....	8.8	11.1
2	47	53	7.3	.....	8.1	10.7
3	50	60	8.3	.....	8.9	12.7
4	45	53	7.2	.....	8.1	11.1
5	45	50	6.8	.....	7.3	11.8
Milk-curd ration:						
1	66	86	10.0	138.3	12.8	45.8
2	60	75	8.5	123.7	11.0	48.5
3	64	81	9.2	129.6	12.6	52.6
4	58	72	7.9	118.3	11.4	45.7
5	58	66	5.8	112.9	8.7	41.6
Swiss-cheese ration:						
1	90	106	10.0	138.1	15.4	55.7
2	78	91	8.7	126.6	12.9	54.7
3	87	97	9.4	128.5	14.8	60.3
4	74	89	8.8	131.3	13.4	50.5
5	64	77	8.2	112.8	15.0	46.5
Milk-curd ration:						
1	117	133	11.5	159.1	15.4	67.1
2	99	112	9.9	136.7	(12.5)	59.7
3	104	122	11.0	149.5	(13.7)	61.9
4	97	108	9.3	131.8	12.4	60.0
5	85	96	8.5	122.2	10.9	47.1
Standardizing ration:						
1	137	150	11.4	.....	15.0	22.6
2	119	123	9.6	.....	12.9	26.7
3	126	140	12.0	.....	14.0	26.1
4	112	122	10.7	.....	13.4	23.4
5	100	110	9.9	.....	12.5	22.7

RATS 6 TO 10

Standardizing ration:						
6	50	56	6.7	.....	7.7	10.4
7	46	52	5.8	.....	6.8	10.7
8	49	58	7.3	.....	8.1	11.4
9	49	56	7.1	.....	8.1	12.2
10	47	55	7.1	.....	7.9	11.0
Swiss-cheese ration:						
6	59	68	6.6	90.0	10.1	34.3
7	57	69	6.4	89.9	8.7	30.0
8	65	80	8.9	119.7	11.8	43.6
9	63	77	8.7	118.9	12.2	41.2
10	60	71	8.2	110.1	11.3	45.1
Milk-curd ration:						
6	75	87	8.0	115.7	12.3	49.5
7	74	87	8.1	115.2	10.7	45.6
8	88	102	9.3	131.8	13.2	58.8
9	85	99	9.2	130.8	13.2	55.6
10	76	89	8.7	124.7	12.7	53.6
Swiss-cheese ration:						
6	92	102	8.1	117.9	13.5	55.8
7	93	103	7.2	109.8	12.2	41.9
8	105	117	9.7	133.5	13.5	57.4
9	104	116	9.9	135.3	15.2	64.7
10	95	104	9.3	128.6	14.3	65.6
Standardizing ration:						
6	108	112	8.2	.....	12.6	19.7
7	110	117	8.8	.....	11.7	20.2
8	124	132	10.8	.....	14.4	24.3
9	121	129	11.3	.....	17.1	30.9
10	111	111	7.8	.....	12.6	31.7

All the nitrogen-metabolism data of this experiment are assembled in table 9, while the digestion coefficients and biological values computed from them are summarized in tables 10 and 11. To conserve space in the tables the intermediate calculations are omitted, the method of calculation having been fully explained in earlier publications from this laboratory.

TABLE 10.—*Coefficients of apparent and true digestibility of the protein of Swiss cheese and of rennet milk curd* [1]

Rat no.	Apparent digestibility				True digestibility			
	Milk-curd protein		Swiss-cheese protein		Milk-curd protein		Swiss-cheese protein	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	90.7	90.3	88.6		99.0	99.5	97.5	
2	91.1	90.9	89.8		99.3	100.0	98.4	
3	90.3		88.4		98.2	99.3	96.8	
4	90.4	90.6	89.8		98.3	99.3	97.8	
5	92.3	91.1	86.7		98.3	99.7	95.3	
6	89.3		88.8	88.5	98.8		98.0	98.4
7	90.7		90.3	88.9	99.5		98.9	97.3
8	90.0		90.1	89.8	98.6		98.8	99.0
9	89.9		89.7	88.8	99.3		98.7	99.2
10	89.8		89.7	88.9	99.3		98.9	99.6
Average	90.53		89.12		99.09		98.17	

[1] For rats 1 to 5, 2 digestion trials were run on the milk-curd ration, while for rats 6 to 10, 2 digestion trials were run on the Swiss-cheese ration.

TABLE 11.—*Biological values of the protein of Swiss cheese and of rennet milk curd*

Rat no.	Milk-curd protein		Swiss-cheese protein		Rat no.	Milk-curd protein		Swiss-cheese protein	
	Trial 1	Trial 2	Trial 1	Trial 2		Trial 1	Trial 2	Trial 1	Trial 2
1	77	71	71		7	74		81	78
2	72	73	71		8	70		76	73
3	71	73	67		9	74		79	72
4	73	71	74		10	74		73	70
5	76	78	72						
6	70		75	67	Average	73.1		73.3	

An illustration of the calculation of a biological value for protein is the following: Rat no. 6 in period 2 consumed 6.59 g daily of the Swiss-cheese ration, containing 90 mg of nitrogen. The average daily excretion of nitrogen in the feces was 10.1 mg, of which 8.3 mg (1.26×6.59) was estimated to be of body origin. Hence, the fecal nitrogen of food origin was equal to $10.1 - 8.3 = 1.8$ mg. The absorbed food nitrogen therefore is equal to $90 - 1.8 = 88.2$ mg. The urinary nitrogen for the period averaged 34.3 mg daily, the sum of 12.5 mg of endogenous nitrogen (19.2×65, the average body weight of the rat for the period) and 21.8 mg of food nitrogen unutilized in metabolism. Of the nitrogen absorbed, $88.2 - 21.8 = 66.4$ mg were retained in the body for both maintenance and growth. The biological value is this figure expressed as a percentage of the absorbed nitrogen, i.e. $(66.4 \div 88.2) \times 100 = 75$.

The digestion coefficients given in table 10 again reveal a lower digestibility of cheese nitrogen than of milk-curd nitrogen. The average coefficients of apparent digestibility are 89.12 and 90.53, respectively. If the fecal nitrogen is reduced by the amount of

metabolic nitrogen estimated to be present in the feces, it is possible to compute coefficients of true digestibility, which average 98.17 and 99.09, respectively, the advantage again resting with the milk-curd protein.

However, the biological values computed for the two protein sources were very nearly the same, averaging 73.1 for milk-curd protein and 73.3 for Swiss-cheese protein (table 11).

These determinations of the biological values of the protein of milk curd and of Swiss cheese indicate that the good showing of the cheese proteins in the paired-feeding experiments, in spite of a distinctly lower digestibility, cannot be explained on the basis of a higher utilization of their absorbed nitrogen in the anabolic chemical reactions of maintenance and growth. The remaining possibility is that the explanation is to be found in the composition of the gains in weight produced on the two types of rations, one containing milk curd as its main source of protein and one containing cheese as its predominant source of protein.

NITROGEN AND ENERGY CONTENT OF PAIRED RATS ON MILK-CURD AND LIMBURGER-CHEESE RATIONS

In order to investigate this possibility, the 16 rats used in the paired-feeding comparison of milk curd and Limburger cheese (table 5) were analyzed after removal of the gastrointestinal tract and contents. The feeding experiment had shown no advantage for either sort of protein in the promotion of gains in weight but did show a clear advantage of the milk-curd protein over the cheese protein in digestibility (table 7). The nitrogen contents of the rat carcasses were determined by the Kjeldahl method, and their gross energy values (heats of combustion) by the Parr oxgyen bomb calorimeter. The results of these determinations are given in table 12.

TABLE 12.—*Nitrogen and energy contents of paired rats on milk-curd and Limburger-cheese rations*

Pair no.	Protein in ration	Body weight of rat minus alimentary tract	Nitrogen content		Energy content	
			Percent	Grams	Per gram	Total
		Grams			*Calories*	*Calories*
1	Milk curd	123	2.57	3.16	2.24	276
	Cheese	130	2.32	3.02	2.43	315
2	Milk curd	144	2.69	3.87	2.53	365
	Cheese	135	2.74	3.70	2.34	316
3	Milk curd	129	2.70	3.48	2.26	292
	Cheese	129	2.55	3.29	2.33	301
4	Milk curd	140	2.68	3.75	2.22	311
	Cheese	137	2.52	3.45	2.42	332
5	Milk curd	109	2.69	2.93	1.89	206
	Cheese	109	2.68	2.92	2.21	241
6	Milk curd	113	2.82	3.19	1.84	208
	Cheese	117	2.36	2.76	2.11	247
7	Milk curd	114	2.65	3.02	2.00	228
	Cheese	112	2.39	2.68	2.51	281
8	Milk curd	94	2.90	2.73	2.06	194
	Cheese	95	2.67	2.54	2.09	199

An analysis according to the Student method of the differences between pair mates with respect to their nitrogen and energy contents is given in table 13.

TABLE 13.—*Analysis according to the Student method of the differences between pair mates with respect to nitrogen and energy contents*

Item	Nitrogen content		Energy content	
	Percent	Grams	Per gram	Total
			Calories	*Calories*
Mean of differences, M [1]	+0.182	+0.221	−0.175	−19.0
Standard deviation, σ	.148	.122	.196	29.9
Ratio $z=M\div\sigma$	1.23	1.81	.89	.64
Probability, P	.0071	.001	.025	.068

[1] The differences between pair mates were given a plus sign when the value for the rat on the milk-curd ration was the larger, and a negative sign when it was the smaller.

In every pair of rats but one, the percentage of nitrogen was higher for the rat on the milk-curd diet, and in all eight pairs the weight of nitrogen was higher. The probabilities that these paired differences were determined only by chance are so low (i.e., 0.0071 and 0.001, respectively) that they may be neglected. It seems certain, therefore, that the difference in the rations produced the difference in nitrogen content of the rats, the milk-curd ration promoting, to a greater extent, the deposition of nitrogen during growth.

The values for the energy contents of the rats presents a different picture, though not so convincing. In every pair but one the energy content of the rat receiving the Limburger-cheese ration, whether expressed in calories per gram of rat or in total calories, was greater than that of the rat raised on the milk-curd ration. The probabilities that chance alone could have produced these results may not be small enough to disregard entirely, although with respect to the gross energy per gram chance would be expected to produce such a one-sided outcome once in about 40 times. In this case at least it may be said with practical certainty that the difference in ration was responsible, the Limburger-cheese ration producing a carcass with the higher gross-energy content presumably because of a greater content of fat.

TABLE 14.—*Estimated gains in nitrogen and gross energy of paired rats on milk-curd and Limburger-cheese rations*

Pair no.	Protein of ration	Body weights minus intestinal tract		Estimated initial content of rats in—		Observed final content of rats in—		Gains in—	
		Initial [1]	Final	Nitrogen	Energy	Nitrogen	Energy	Nitrogen	Energy
		Grams	*Grams*	*Grams*	*Calories*	*Grams*	*Calories*	*Grams*	*Calories*
1	Milk curd	42	123	1.14	63	3.16	276	2.02	213
	Cheese	46	130	1.25	69	3.02	315	1.77	246
2	Milk curd	53	144	1.44	80	3.87	365	2.43	285
	Cheese	50	135	1.36	75	3.70	316	2.34	241
3	Milk curd	44	129	1.20	66	3.48	292	2.28	226
	Cheese	47	129	1.28	71	3.29	301	2.01	230
4	Milk curd	49	140	1.33	74	3.75	311	2.42	237
	Cheese	47	137	1.28	71	3.45	332	2.17	261
5	Milk curd	33	109	.90	50	2.93	206	2.03	156
	Cheese	35	109	.95	53	2.92	241	1.97	188
6	Milk curd	37	113	1.01	56	3.19	208	2.18	152
	Cheese	36	117	.98	54	2.76	247	1.78	193
7	Milk curd	36	114	.98	54	3.02	228	2.04	174
	Cheese	37	112	1.01	56	2.68	281	1.67	225
8	Milk curd	31	94	.84	47	2.73	194	1.89	147
	Cheese	30	95	.82	45	2.54	199	1.72	154

[1] Estimated.

In order to eliminate in these comparisons the results of variable initial contents of nitrogen and energy among the paired rats, the gains in nitrogen and in energy were computed from the observed final contents and from estimates of the initial contents based on the initial weights. The initial weights of the rats were corrected to the weights minus the intestinal tract by the use of Donaldson's tables (*4*). The remainder of the carcass was then assumed to contain 2.72 percent of nitrogen and 1.505 calories of gross energy per gram on the basis of the results of Mitchell and Carman (*9*). The computation of the gains of nitrogen and energy is given in table 14.

The differences between pair mates in the storage of nitrogen averaged 232 mg in favor, in each pair, of the milk-curd ration. The standard deviation of differences was 113 mg, and the probability that this outcome is a result of chance is only 0.0005, clearly negligible. The differences between pair mates in energy storage averaged 18.5 calories, and in all pairs but one favored the Limburger-cheese ration. However, the standard deviation of differences is 27.9 calories and the desired probability is 0.063. These results are therefore merely highly suggestive of a ration effect.

It appears that the less digestible Limburger-cheese nitrogen promoted a smaller storage of nitrogen in the paired-feeding experiment than the same intake of milk-curd nitrogen. Nevertheless, the gains in weight were just as large, due in all probability to a greater deposition of fat in the rats on the cheese ration.

TABLE 15.—*Estimation of the biological values of the protein of milk curd and Limburger cheese when fed to paired rats*

Pair no.	Protein of ration	Average body weight	Total intake of digestible nitrogen	Total endogenous nitrogen	Nitrogen stored in tissues	Total nitrogen utilized	Biological value of nitrogen
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
1	Milk curd	90.4	5.25	0.99	2.02	3.01	57
	Cheese	96.5	5.24	1.06	1.77	2.83	54
2	Milk curd	106.0	5.34	1.17	2.43	3.60	67
	Cheese	100.6	5.30	1.11	2.34	3.45	65
3	Milk curd	95.7	5.44	1.05	2.28	3.33	61
	Cheese	96.0	5.34	1.06	2.01	3.07	57
4	Milk curd	104.1	6.13	1.15	2.42	3.57	58
	Cheese	100.5	5.96	1.11	2.17	3.28	55
5	Milk curd	79.5	4.45	.87	2.03	2.90	65
	Cheese	78.5	4.29	.86	1.97	2.83	66
6	Milk curd	84.5	5.16	.93	2.18	3.11	60
	Cheese	84.0	5.00	.92	1.78	2.70	54
7	Milk curd	81.9	4.67	.90	2.04	2.94	63
	Cheese	81.0	4.62	.89	1.67	2.56	55
8	Milk curd	67.7	4.25	.75	1.89	2.64	62
	Cheese	68.5	4.23	.75	1.72	2.47	58

It is possible from the data at hand to determine more or less certainly whether the smaller storage of nitrogen on the cheese ration was entirely accounted for by the smaller digestibility of the cheese nitrogen. In table 15 will be found computations similar in import to those leading to the determination of biological values from the data of metabolism experiments. The only explanation necessary relates to the computation of the endogenous nitrogen. The values given for this item cover the entire period of the feeding experiment. They are based upon the average weight of the rat for the feeding

period and an average value, taken from metabolism experiments, of 22 mg of endogenous nitrogen per day per 100 g of body weight. It should be said of the biological values given in the last column that they may be less than those obtained in a short metabolism experiment if, during the prolonged feeding period, the rat has shed appreciable amounts of hair and scurf. In such case the nitrogen stored in the tissues is really greater than the difference between the initial and final nitrogen content of the rat.

From the table it is evident that in all pairs but one the computed biological value of the dietary protein is smaller for the Limburger cheese than for the milk curd, the averages being, respectively, 58 and 62. The average difference is 3.63 and the standard deviation of differences 2.49. The probability that chance alone would account for the paired differences is only 0.0031. It seems, therefore, that the biological value of Limburger-cheese protein is somewhat less (about 6 percent) than that of its mother substance, the milk-curd protein. Such a difference was not detected in metabolism experiments affording a comparison of the protein of Swiss cheese and of milk curd (table 11), though this fact cannot be considered inconsistent with the finding for Limburger cheese because of the more extensive ripening to which the latter cheese was evidently subjected.

If the protein of milk curd, casein, has suffered a deterioration in nutritive value during the conversion of the curd into Limburger cheese, then it may be expected that the amino acid deficiency of the casein would be altered. It has been demonstrated that casein is deficient in the sulphur-containing amino acid, cystine (*11*). If, during the ripening process, cystine is destroyed more rapidly than other amino acids indispensable in nutrition, then the cystine deficiency of the cheese protein will be greater than the cystine deficiency of casein, so that a greater improvement in its biological value will result when it is supplemented with an excess of cystine. On the other hand, if some other indispensable amino acid is destroyed more rapidly than the cystine, then the resulting cheese protein will possess a smaller deficiency of cystine, or even no cystine deficiency at all.

CYSTINE DEFICIENCIES OF MILK-CURD AND LIMBURGER-CHEESE PROTEINS

In measuring the cystine deficiencies of the protein of milk curd and the protein of Limburger cheese, the paired-feeding technic was modified. The ordinary technic as used in the preceding experiments is for some purposes open to the criticism that the better of two rations being compared is in a sense being penalized, because the rats receiving it are growing at the faster rate and are correspondingly increasing their maintenance requirements over those of their pair mates. Hence, of the equal intakes of food, a smaller proportion of the better ration will be available for growth over and above the maintenance requirements. Osborne and Mendel (*11, p. 9*) have discussed the same situation and have concluded: "The only strict basis for comparison is afforded by experiments in which the animals receive the same amount of food during the same period of time and make the same gain in weight."

This principle was incorporated into the paired-feeding technic, as illustrated by the milk-curd experiment. Several kilograms of a milk-curd ration containing 8 percent of protein, and otherwise

similar in make-up to the rations used in the preceding experiments, were prepared, thoroughly mixed in an electric mixer, and divided into two equal portions. One portion was used unchanged, while to the other portion was added 0.3 percent of cystine, equivalent to 3.75 percent of the protein. This proportion of cystine is more than enough to supplement the casein. The two rations were then fed to a series of pairs of rats in the ordinary way until differences in weight developed between pair mates. Then the rat in each pair that received the cystine diet was fed a combination of this diet and of a low-nitrogen diet similar to it except that it contained no cystine and that starch was substituted for the dried milk curd. The total amount of the two rations fed to the rats receiving cystine was kept the same as the amount of food consumed by their pair mates on the unsupplemented milk-curd ration, while the proportion between the amounts of the two combined rations was so adjusted that the gains in weight of pair mates were equalized as closely as possible. Thus, as the rats on the cystine ration increased in weight beyond their pair mates, they were given a greater proportion of the low-nitrogen diet, and vice versa. From 3 to 5 drops of tiki-tiki extract were given daily to each rat in order to maintain the appetite.

TABLE 16.—*Supplementing effect of cystine on the protein of rennet milk curd during a feeding period of 49 days when fed to paired rats*

Item	Pair 1, females		Pair 2, females		Pair 3, females		Pair 4, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight.....grams..	122	121	129	128	115	121	128	125
Initial weight.....do....	46	44	50	50	49	49	50	50
Gain.....do....	76	77	79	78	66	72	78	75
Body length.....centimeters..	18.4	18.3	18.4	18.4	18.3	18.6	18.2	18.1
Food intake.....grams..	443	443	466	466	428	428	447	447
Nitrogen intake.....do....	5.71	4.41	6.01	4.23	5.53	3.86	5.77	4.34
Ratio of nitrogen intakes.....	1.29	1	1.42	1	1.43	1	1.33	1

Item	Pair 5, females		Pair 6, females		Pair 7, males		Pair 8, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight.....grams..	138	133	126	120	153	152	138	142
Initial weight.....do....	48	46	49	45	57	55	53	56
Gain.....do....	90	87	77	75	96	97	85	86
Body length.....centimeters..	18.8	18.9	18.0	18.2	19.5	19.4	18.9	19.1
Food intake.....grams..	447	447	451	451	500	500	488	488
Nitrogen intake.....do....	5.77	5.15	5.82	4.50	6.45	4.96	6.30	5.22
Ratio of nitrogen intakes.....	1.12	1	1.29	1	1.30	1	1.21	1

The results of this type of an experiment on milk-curd protein are given in table 16. On the same intakes of food the gains in weight of pair mates were kept very nearly the same (except in one pair in which the difference amounted to 6 g) simply by varying the nitrogen intake. A considerably smaller amount of nitrogen from the mixture of casein and cystine promoted the same gains in weight. From the ratios between the nitrogen intakes of pair mates, given in the last line of the table, it appears that 1 g of nitrogen from the casein-cystine mixture

produced the same nutritive effect as 1.12 to 1.43 g of nitrogen (averaging 1.30 g) from the unsupplemented casein. Taking the biological value of casein as 73, that of the supplemented casein would be $73 \times 1.3 = 95$,[3] on the reasonable assumption that cystine did not affect the digestibility of the casein. Mitchell (*8*) has reported metabolism data on a rat, indicating a biological value of 75 for casein, and a value of 92 for casein fully supplemented with cystine.

Three pairs of rats in this experiment, nos. 1, 7, and 8, were analyzed at the end of the feeding period to determine whether the composition of the carcasses had been affected by the difference in dietary treatment. Only the nitrogen and energy contents of the empty carcasses were determined. The results are given in table 17.

TABLE 17.—*Nitrogen and energy contents of empty carcasses of three pairs of rats used in experiments for which data are recorded in table 16*

Pair no.	Dietary protein	Nitrogen content	Energy content per gram
		Percent	*Calories*
1	Milk curd	3.34	2.07
	Milk curd+cystine	3.30	2.18
7	Milk curd	3.03	2.30
	Milk curd+cystine	3.02	2.64
8	Milk curd	3.18	2.31
	Milk curd+cystine	3.28	2.22

The differences between pair mates were slight and did not show any consistency, so that it may be concluded that the similar gains of pair mates signified similar nitrogen retention. For these six rats computations of the biological value of the dietary protein were made, according to the method used in a preceding experiment (tables 14 and 15), with the results given in table 18. The unsupplemented milk-curd protein exhibited in this test biological values of 73, 73, and 70, which are of the same magnitude as those determined by the nitrogen-balance studies (table 11). The supplemented casein gave biological values of 93, 95, and 88, averaging 92. The results of the feeding experiment led to an estimate of 95.

TABLE 18.—*Estimation of the biological values of the protein of milk curd with and without a cystine supplement when fed to paired rats*

Pair no.	Supplement	Total intake of digestible nitrogen	Total endogenous nitrogen	Total nitrogen stored in tissues	Total nitrogen utilized	Biological value
		Grams	*Grams*	*Grams*	*Grams*	*Percent*
1	None	5.15	0.95	2.83	3.78	73
	Cystine	3.98	.95	2.75	3.70	93
7	None	5.80	1.12	3.09	4.21	73
	Cystine	4.43	1.12	3.09	4.21	95
8	None	5.66	1.06	2.91	3.97	70
	Cystine	4.71	1.08	3.06	4.14	88

[3] It must be admitted that part of this increase in the biological value of casein accompanying the addition of cystine may be due to the fact that the supplemented casein was fed at a lower percentage of the diet than the unsupplemented. This in itself would tend to increase the biological value, although all proteins do not show this effect (*7*).

The Limburger cheese was tested by the same methods applied to a study of the cystine deficiency of milk-cured protein. Eight pairs of rats were used in a preliminary test and were carried for 2 weeks on equalized intakes of the supplemented and unsupplemented rations containing equal percentages of nitrogen. The purpose of this test was to determine whether the protein of Limburger cheese was deficient in cystine. At the end of 2 weeks of feeding such a deficiency was clearly established. In seven pairs, the greater gains were made by the rat receiving the cystine-supplemented diet, while in one pair the gains were equal. The average difference in gain between pair mates was 3.50 g, and the standard deviation of differences was 2.46 g. The probability that the average difference was the result merely of a fortuitous combination of factors common to both rats of a pair is only 0.0036, certainly small enough to be negligible.

TABLE 19.—*Supplementing effect of cystine on the protein of Limburger cheese during a feeding period of 45 days when fed to paired rats*

Item	Pair 1, females		Pair 2, males		Pair 3, females		Pair 4, males	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight.....grams..	85	86	98	97	95	98	110	108
Initial weight.....do....	42	42	40	39	44	47	47	45
Gain.....do....	43	44	58	58	51	51	63	63
Body length.....centimeters..	16.5	16.8	17.0	17.2	16.9	17.0	17.4	17.6
Food intake.....grams..	305	305	290	290	292	292	282	282
Nitrogen intake.....do....	4.14	3.61	3.93	3.47	3.97	3.67	3.83	3.20
Ratio of nitrogen intakes.....	1.15	1	1.13	1	1.08	1	1.20	1

Item	Pair 5, males		Pair 6, males		Pair 7, females		Pair 8, females		Pair 9, females	
	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine	No cystine	Cystine
Final weight.....grams..	87	87	100	100	112	112	100	99	102	101
Initial weight.....do....	38	39	43	42	48	48	46	43	32	32
Gain.....do....	49	48	57	58	64	64	54	56	70	69
Body length.....centimeters..	17.8	17.8	17.3	17.7	17.8	18.3	17.3	17.4	17.4	17.5
Food intake.....grams..	255	255	296	296	375	375	319	319	328	328
Nitrogen intake.....do....	3.46	3.33	4.02	3.57	5.08	4.51	4.33	3.75	4.45	3.95
Ratio of nitrogen intakes.....	1.04	1	1.13	1	1.13	1	1.15	1	1.13	1

A cystine deficiency in the protein of Limburger cheese having been established, the extent of this deficiency was quantitatively assessed by the modified paired-feeding technic. Nine pairs of rats were used in the experiment. The results obtained are summarized in table 19. A very close equalization of the gains of pair mates was secured in this test, but a relatively greater proportion of the cheese-cystine nitrogen was required than of the casein-cystine nitrogen in the preceding test. The ratios of nitrogen intakes from the unsupplemented and the supplemented cheese rations ranged from 1.04 to 1.20 to 1, and averaged 1.13 to 1. If the biological value of the Limburger-cheese protein may be taken as 6 percent less than that of casein (Table 11), namely 69, then the addition of cystine increased it only 13 percent, giving a value of 78. This is appreciably less than the value of 92 to 95 given by milk-curd protein plus cystine. It may be concluded, therefore,

that the ripening of the curd in the manufacture of Limburger cheese lowered the biological value of the protein by reactions involving the destruction of cystine to some extent, but to a greater extent the destruction of some one or more of the other amino acids indispensable in animal nutrition. We may infer that cystine has been involved to some extent in these reactions both because it is still the limiting deficiency of the cheese protein, and because the biological value of the cheese protein appears to be somewhat less than that of the curd protein. We may infer that other indispensable amino acids are also involved, because the improvement in biological value induced by the addition of cystine is considerably less than the improvement noted in the case of casein. In other words, a second limiting amino acid deficiency is manifest in the supplemented cheese protein at a lower biological value than in the supplemented curd protein.

DISCUSSION

The most general and clear-cut finding of the feeding and metabolism experiments reported in this paper on four varieties of cheese (Cheddar, Roquefort, Swiss, and Limburger) was that in the ripening process to which milk curd is subjected in the manufacture of cheese, the digestibility of the protein of the curd is depressed 1 or 2 percent. However, the capacity of the cheese protein to promote gains in weight was not lowered. In fact, in the case of Swiss cheese, its gain-promoting capacity was definitely, though slightly, increased over that of milk curd. In metabolism experiments on 10 rats no differences in the biological values of rennet curd and of Swiss cheese could be distinguished. Hence, the depressed digestibility of the protein of this cheese could be reconciled with its increased capacity to promote gains in weight in young rats, most probably on the assumption that the chemical composition of the weight increases on cheese portein showed less protein and more fat than those produced by milk-curd protein.

This assumption was tested for Limburger cheese, the protein of which was found to be as efficient as milk-curd protein in promoting gains in weight, within the limits of accuracy of the paired-feeding method, although it also was distinctly less digestible. By analyzing the carcasses of the rats at the conclusion of the paired-feeding tests, and from average analyses of rats at the initial weight, computing the nitrogen and gross energy content of the gains in weight produced, it was clearly shown that the assumption was correct. It was also shown that in the case of Limburger cheese the biological value of the protein was slightly depressed, about 6 percent, below that of the casein of milk curd. The contrast in this respect between this protein and that of Swiss cheese is probably related to the more extensive ripening which the Limburger cheese undergoes.

The fact that equal gains in weight put on in the same periods on the same amounts of two rations may differ appreciably in composition, indicates that a gram gain in weight in experimental rats possesses a variable nutritive significance, although in the current interpretation of feeding experiments on rats, less well controlled than were these, the nutritive significance of gains in weight are assumed to vary only with their size. This experiment on Limburger

cheese confirms the conclusion of Mitchell and Carman (*9, p. 410*), expressed 8 years ago:

> Therefore, the gain in weight of an animal, no matter how carefully experimental conditions may be prescribed, is not a definite measure of the nutritive effect of the ration fed, but is subject to considerable variation due to the operation of unknown factors.

It proves beyond doubt that the exact interpretation of feeding experiments, no matter how well controlled they may be, requires an investigation of the chemical composition of the carcasses produced by the experimental feeding.

The analyses of the carcasses of experimental rats in these studies have permitted the computation of the biological value of dietary protein by another method than that based on the metabolism data secured over a short period. The former biological values may be less than the latter on account of the loss of hair and scurf during a long feeding period, but in one case, involving a study of milk-curd protein, the two computations led to practically identical results.

The nutritive value of Limburger-cheese protein, and presumably of that of the proteins of other cheeses in which the chemical changes of ripening are no more extensive, is limited by a deficiency of cystine, in a similar manner, though not to so great an extent, as the paracasein of milk curd. In studying the quantitative aspects of the cystine deficiency of cheese and milk curd, a modification of the paired-feeding method was used, involving a principle enunciated by Osborne and Mendel in one of their earlier series of feeding experiments, namely, that in the comparison of the growth-promoting values of proteins the experimental animals should receive "the same amount of food during the same period of time and make the same gain in weight", the only variable being the intake of protein.

CONCLUSIONS

The digestibility of the protein of Roquefort, Swiss, and Limburger cheese is from 1 to 2 percent less than that of rennet milk curd.

The biological value of the protein of Swiss cheese is practically the same as that of rennet curd, i.e., 73, while the biological value of the protein of Limburger cheese is about 6 percent less.

In Limburger cheese, and presumably in all cheeses in which ripening has proceeded no further, cystine is the amino acid limiting its biological utilization, as is true of casein itself. However, the biological value of the protein of Limburger cheese is not increased as much by cystine supplementation as is the biological value of casein.

Cystine destruction is involved in the ripening reactions in the preparation of Limburger cheese, as is also one other, at least, of the amino acids indispensable in animal nutrition.

The accurate interpretation of the gains in weight of animals secured in feeding experiments, no matter how well controlled, requires an investigation of their nutrient content.

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THE EFFECT OF EGG PRODUCTS ON THE WHIPPING PROPERTIES OF ICE-CREAM MIXES MADE FROM BUTTER AND DRIED SKIM MILK[1]

By C. C. WALTS, *fellow in dairy husbandry*, and C. D. DAHLE, *professor of dairy manufactures, Department of Dairy Husbandry and Bacteriology, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

Several investigators (*11*, *12*, *18*)[2] have demonstrated that the whipping properties of ice-cream mixes can be markedly improved by the addition of egg yolk to the mix, and considerable research (*2*, *3*, *4*, *5*, *21*, *22*) has been conducted to determine why certain products produce the beneficial effects noted. At present, however, these researches have not shown conclusively why egg yolk exerts so decided an effect upon the whipping properties of the ice-cream mixture.

The experiments reported in this paper were made to determine the effect produced by the addition of various egg products to ice-cream mixes made with unsalted butter, dried skim milk, and water.

REVIEW OF LITERATURE

Button (*2*, *3*) separated dried yolk into yolk protein, egg oil, lecithin, and cholesterol. He considered that the egg oil and the lecithin cause the slow initial rise of overrun when egg yolk is used in the mix. The improvement in whipping properties he attributed to the egg-yolk protein. He found egg albumin to be detrimental to the whipping qualities of the mix; it exhibited no stabilizing properties and injured both the texture and the flavor of the ice cream. Dried egg yolk and dried whole egg did not contribute a stabilizing effect but did improve the texture, particularly in low-solid mixes. Less time was required to obtain 100 percent overrun when dried egg yolk and dried whole eggs were added to low-solid mixes. Flavors due to egg products were more pronounced in low-solid mixes than in high-solid mixes.

Tracy and Ruehe (*22*) and Caulfield and Martin (*4*) found that the addition of egg or soybean lecithin to ice-cream mixes did not improve their whipping properties and that soybean lecithin acted as a deterrent to overrun. Dahle (*5*) also reported the failure of soybean lecithin in amounts ranging from 0.1 to 0.4 percent to exert any beneficial effect on the whipping properties of a mix.

Martin (*10*), after studying the effect of certain acetone and ether extracts from egg yolk, reached no conclusion as to why egg yolk was beneficial to the whipping properties of an ice-cream mix.

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[2] Reference is made by number (italic) to Literature Cited, p. 976.

Sommer and Horrall (*21*) were unable to secure any improvement in the whipping properties of ice-cream mixes containing egg lecithin, egg oil, or egg-yolk protein, either separately or combined.

Richardson (*20, p. 435*) gives the composition of egg yolk, according to Gobley, as follows:

	Percent		Percent
Glycerophosphoric acid	1. 2	Cholesterol	0. 4
Vitellin	15. 8	Fats	20. 3
Nuclein	1. 5	Coloring matters	. 5
Cerebrin	. 3	Salts	1. 0
Lecithin	7. 2	Water	51. 8

METHOD OF PROCEDURE

All the ice-cream mixes were made from Extra grade, spray-process, dried skim milk and fresh, good quality unsalted butter, except certain control mixes, in which fresh 40 percent cream was used as the source of fat. Cane sugar was employed as the sweetening agent. The gelatin tested 220 Bloom grams. The egg lecithin and soybean lecithin used were obtained from recognized sources.

Sixty-pound mixes, made in 10-gallon cans, had the following composition: 12 percent fat, 11 percent serum solids, 15 percent sugar, 0.25 to 0.37 percent gelatin, and 38.25 percent total solids. All mixes were pasteurized at 150° F. for 30 minutes. The mixes were viscolized at 2,000 pounds pressure at 150°, a single-stage valve being used unless otherwise noted. The mixes were cooled quickly over a surface cooler to approximately 40° F. and aged 20 to 22 hours at 40°. For freezing the mixes a 40-quart brine freezer was used. The brine temperatures ranged from −5° to −10° F. but were held constant during a series of freezings. The temperature of the ice cream and the percentage of overrun were recorded at 1-minute intervals until 100 percent overrun had been obtained. An ammeter device was used to aid the freezer operator in turning off the brine. The freezer was cooled by freezing a preliminary batch of regular stock mix before the experimental mixes were frozen. Samples of ice cream were drawn for scoring at exactly 100 percent overrun except in the few cases mentioned where this overrun was not obtainable.

After hardening 3 to 5 days at −10° F. the ice cream was scored independently by three judges. It will be noted that the scores reported in the tables contain decimals. These are due to the averaging of the scores rendered by the different judges.

EFFECT OF DIFFERENT PRODUCTS ON WHIPPING PROPERTIES OF THE MIX AND QUALITY OF THE ICE CREAM

DRIED EGG YOLK

The effect of dried egg yolk upon the whipping properties of a mix was first studied. Control mixes were made with cream or unsalted butter, dried skim milk, and water; experimental mixes were made with cream or unsalted butter, dried skim milk, and water to which was added 0.5 percent of dried egg yolk. Viscolization in these experiments was accomplished with a two-stage valve at the pasteurization temperature. The data obtained are presented in table 1.

TABLE 1.—*Effect of egg yolk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3–5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	Minutes				
Cream; dried skim milk; water	8.15	43.58	23.50	5	72.08
Cream; dried skim milk; water; 0.5 percent dried egg yolk	7.50	43.27	23.50	5	71.77
Butter; dried skim milk; water	10.25	43.00	23.50	5	71.50
Butter; dried skim milk; water; 0.5 percent dried egg yolk	7.65	42.84	23.50	5	71.34

The results in table 1 show that the addition of 0.5 percent of dried egg yolk to the butter-dried skim milk mixture effected a minimum saving of 2.6 minutes in the time required to obtain 100 percent overrun. In additional experiments herein reported, as much as 8 minutes saving was noted when 0.5 percent of dried egg yolk was added to butter-dried skim milk mixes. The same amount of egg yolk added to mixes made with cream and dried skim milk resulted in a saving of a little over half a minute. Thus the egg yolk exerted a much more beneficial effect upon the whipping properties of butter-dried skim milk mixes than upon those of cream-dried skim milk mixes.

Ice cream containing cream and egg yolk scored higher than that made from butter and egg yolk.

DRIED DOMESTIC V. CHINESE EGG YOLK; DRIED WHOLE EGG

A study was made of the effect of domestic and Chinese dried egg yolk and of domestic dried whole egg upon the whipping properties of mixes made with butter, dried skim milk, and water. Five tenths percent of the various egg products was added to the experimental mixes. The results are given in table 2.

TABLE 2.—*Effect of whole egg and various kinds of egg yolk or dried whole egg, 0.5 percent in all cases, on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3–5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	Minutes				
Butter; dried skim milk; water	16.50	35.00	23.66	5	63.66
Butter; dried skim milk; water; dried Chinese egg yolk	8.75	35.00	24.20	5	64.20
Butter; dried skim milk; water; dried domestic egg yolk, brand A	9.00	35.33	24.50	5	64.83
Butter; dried skim milk; water; dried domestic egg yolk, brand B	8.50	33.33	24.50	5	62.83
Butter; dried skim milk; water; dried whole egg	9.20	33.33	24.08	5	62.41

An examination of table 2 reveals little difference in the effect of domestic and Chinese egg yolk upon the whipping properties of mixes made with butter and dried skim milk. Both types of yolk materially shortened the time required to obtain the desired yield. The maximum saving in time thus obtained was 8 minutes.

The dried whole egg also exerted a beneficial effect upon the whipping properties of the mix, but not to the same extent as did the dried egg yolk.

As the butter was somewhat old the scores of the ice cream in these experiments were all low.

SWEETENED FROZEN YOLK AND DRIED YOLK

The effect of sweetened frozen egg yolk as compared with that of dried egg yolk was studied. Mixes were made with butter, dried skim milk, and water to which 1 percent of previously frozen egg yolk containing 10 percent of sucrose was added. Other mixes were made of the same ingredients except that instead of frozen egg yolk 0.5 percent of dried egg yolk was added. The results are presented in table 3.

TABLE 3.—*Effect of sweetened frozen egg yolk and dried egg yolk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3-5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	Minutes				
Butter; dried skim milk; water	13	43.00	23.50	5	71.50
Butter; dried skim milk; water; 1 percent sweetened frozen egg yolk [a]	9	43.00	23.50	5	71.50
Butter; dried skim milk; water; 0.5 percent dried egg yolk [a]	6	42.66	23.50	5	71.16

[a] The amount of egg-yolk solids in the sweetened frozen yolk was equivalent to the egg-yolk solids in the dried egg yolk.

Table 3 shows that dried egg yolk had a more beneficial effect on the whipping properties of the mix than did the frozen egg yolk. A saving of 7 minutes in the time required to obtain the desired yield was noted in the case of the mixes containing dried egg yolk, whereas only 4 minutes saving was obtained in the case of the mixes containing sweetened frozen yolk.

Since the actual amount of egg-yolk solids in the sweetened frozen egg yolk was equivalent to the egg-yolk solids in the dried egg yolk, the poorer results obtained with sweetened frozen egg yolk may have been due to the previous freezing of the sweetened yolk or to the presence of sugar in the frozen yolk.

Urbain and Miller (*23*) consider that the lecithin of egg yolk separates out on freezing but that this can be partially prevented by adding certain sugars before freezing the yolk. Price (*19*) noted that the whipping properties of mixes containing frozen egg yolk were inferior to those of mixes containing frozen egg yolk which had previously been frozen with invert sugar. He attributed the difference

to a lesser separation of lecithin in the sweetened yolk during the freezing of the yolk.

The score of the ice cream made with sweetened frozen yolk, as shown in table 3, was the same as the score of the control ice cream, but the ice cream containing the dried yolk scored a trifle less than the control.

CERTAIN EGG-YOLK CONSTITUENTS

In order to study the effect of the various compounds making up egg yolk upon the whipping properties of butter-dried skim milk mixes, dried egg yolk was separated into certain component parts. The egg-yolk salts were obtained by two methods. According to the first method, 4 pounds of dried egg yolk was dialyzed in large collodion bags suspended in distilled water. The dialyzate was condensed in a Pyrex-glass condensing apparatus under 20 to 25 inches of vacuum. An aliquot of the condensate was used in an ice-cream mix. The second method consisted of the ultrafiltration of 0.3 pound of dried egg yolk dissolved in 2 liters of distilled water, which was allowed to stand for a time at 40° F. This amount of egg yolk is equal to approximately 0.5 percent of the weight of the ice-cream mixes made. The ultrafilters consisted of three no. 50 Whatman filter papers previously impregnated with one layer of collodion. These papers were placed in the bottom of a large Büchner funnel and the outer edges were cemented to the sides of the funnel by a thick layer of paraffin. An extra heavy suction flask was used and approximately 25 inches of vacuum were employed in the filtration. Before the egg-yolk liquid was added to the ultrafilter it was filtered through a coarse filter paper in a 40° F. refrigerated room. This first filtrate was subsequently ultrafiltered. The ultrafiltrate was free from colloids and gave strong tests for chlorides. The entire ultrafiltrate was added to a mix made with butter, dried skim milk, and water, allowance being made for the added water of the ultrafiltrate. The residual egg yolk containing the proteins, fat, and phospholipids was used in another ice-cream mix. It was thought that this latter method of securing the egg-yolk salts was less likely to cause alterations in the mix. When the dialysis method was used no preservative was employed and a slight bacterial decomposition occurred during the rather long dialyzing period.

The vitellin and lecithin of the egg yolk were separated by methods given by Richardson (*20*). The lecithin was not separated from the fats, cholesterol, and glycerophosphoric acid but was left with these compounds in order that their combined effect might be observed, since the action of lecithin in purer form was to be studied later.

Mixes were prepared from butter, dried skim milk, and water, and the various egg products were added. Control mixes were made from cream or butter and dried skim milk. The results of these experiments are shown in table 4.

Table 4 indicates that of the various fractions separated from egg yolk, vitellin, which composes the greater portion of the egg-yolk proteins, was detrimental to the whipping properties of the mix. The egg-yolk salts exerted a somewhat beneficial effect. The portion of the egg yolk containing the phospholipids—the portion in which lecithin is most abundant—was likewise beneficial. The part of the yolk containing the salts and proteins in combination was only slightly so.

TABLE 4.—*Effect of certain constituents of egg yolk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3–5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	Minutes				
Cream; dried skim milk; water	9.85	43.33	24.16	5	72.49
Butter; dried skim milk; water	13.75	41.33	24.33	5	70.66
Cream; dried skim milk; water; 0.5 percent dried egg yolk	8.00	43.33	24.50	5	72.83
Butter; dried skim milk; water; 0.5 percent dried egg yolk	8.40	40.16	24.50	5	69.66
Butter; dried skim milk; water; egg-yolk salts	12.15	37.33	24.16	5	66.49
Butter; dried skim milk; water; vitellin from egg yolk	14.00	36.66	24.00	5	65.66
Butter; dried skim milk; water; ether-soluble extract from egg yolk [a]	12.50	35.00	24.00	5	64.00
Butter; dried skim milk; water; ether-extracted egg-yolk residue [b]	13.00	35.33	24.00	5	64.33

[a] This extract contained the fats, cholesterol, lecithin, and glycero-phosphoric acid.
[b] The residue from the extracted egg yolk mentioned in footnote 1 was composed chiefly of the egg-yolk proteins and salts.

Sommer (*21, p. 309–310*) states:

> Further, it has been found by North that egg yolk added to milk greatly increases the electric charge on the fat globules. The absorption of the lecithin-protein complex on the fat globules in an ice-cream mix may be expected to increase the negative potential on the globules. The higher charge on the globule will prevent fat clumping to a greater extent. Both the better adhesion of the serum to the fat globules and the decreased clumping are conducive to a higher whipping ability.

Although the data are not shown in the tables, there was invariably less fat clumping in the egg-yolk mixes than in the mixes without egg yolk.

EGG AND SOYBEAN LECITHINS

Studies were made of the effect of egg lecithin and of soybean lecithin on the whipping properties of mixes made from butter, dried skim milk, and water. To these mixes various amounts up to 0.2 percent of egg and soybean lecithin were added. The lecithin was incorporated into the mix by thoroughly mixing it with about a pound of melted butter. All of the mixes were viscolized with a two-stage valve at 2,000 pounds pressure at the pasteurization temperature. The results are shown in table 5.

It will be noted in table 5 that the addition of egg lecithin up to 0.2 percent exerted a beneficial effect on the whipping properties of the ice-cream mix made with butter, dried skim milk, and water. A saving of 4.83 minutes in the time required to secure 100 percent overrun was obtained when 0.17 percent of egg lecithin was added. On the other hand, soybean lecithin in amounts ranging from 0.1 to 0.2 percent had a detrimental effect on the whipping properties and the percentage of overrun obtained. The larger the percentage of soybean lecithin used, the more noticeable was this effect.

The flavor of the ice cream was lowered with each increment increase of commercially prepared egg or soybean lecithin.

TABLE 5.—*Effect of varying amounts of egg lecithin and soybean lecithin on the whipping properties of the mix and quality of the ice cream*

EGG LECITHIN

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3–5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	Minutes				
Butter; dried skim milk; water	11. 43	42. 50	23. 33	5	70. 83
Butter; dried skim milk; water; 0.01 percent egg lecithin	9. 30	42. 50	23. 33	5	70. 83
Butter; dried skim milk; water; 0.05 percent egg lecithin	9. 00	41. 47	23. 33	5	69. 80
Butter; dried skim milk; water; 0.10 percent egg lecithin	8. 36	36. 00	23. 33	5	64. 33
Butter; dried skim milk; water; 0.15 percent egg lecithin	7. 55	30. 33	23. 33	5	58. 66
Butter; dried skim milk; water; 0.17 percent egg lecithin	6. 60	20. 66	23. 33	5	48. 99
Butter; dried skim milk; water; 0.20 percent egg lecithin	8. 55	19. 66	23. 33	5	47. 99

SOYBEAN LECITHIN

Butter; dry skim milk; water	9. 50	42. 16	23. 16	5	70. 32
Butter; dried skim milk; water; 0.1 percent soybean lecithin [a]	12. 00	41. 33	23. 83	5	70. 16
Butter; dried skim milk; water; 0.15 percent soybean lecithin [a]	[b] 14. 00	41. 00	22. 50	5	68. 50
Butter; dried skim milk; water; 0.17 percent soybean lecithin [a]	[c] 13. 00	40. 33	22. 16	5	67. 49
Butter; dried skim milk; water; 0.2 percent soybean lecithin [a]	[d] 12. 00	39. 00	21. 50	5	65. 50

[a] Amounts of lecithin used were calculated on the basis of the soybean lecithin being 55 percent pure according to the method used for determining the purity of egg lecithin.
[b] Maximum percentage of overrun obtainable, 61.
[c] Maximum percentage of overrun obtainable, 57.
[d] Maximum percentage of overrun obtainable, 54.

Why the results obtained with the two types of lecithin were so different is not known. The fact that the fatty acids found in soybean lecithin are not all the same as those in egg lecithin (*8*) may have influenced the results to some extent. Moreover, commercial soybean lecithin differs from egg lecithin in that it is a mixture of vegetable lecithin and a neutral oil, the oil being added chiefly to increase the solubility and stability of the lecithin in those products in which it is used. Vegetable lecithin is understood to have associated with it a carbohydrate group [3] and this might conceivably influence its colloidal behavior. Richardson (*20*) and Osborne and Campbell (*13*) consider that egg lecithin is present both free and in combination with the egg-yolk proteins. Sommer (*21, p. 308–09*) states:

> It suggests the hypothesis that the egg lecithin, because of its fatty nature (glycerol and two fatty acid radicals in the molecule), will dissolve in the fat globules but will remain oriented at the surface because of the other groups in the lecithin molecule having polar properties (affinity for water). If the egg lecithin is in combination with egg proteins, then this would result in the formation of an adsorption film around the fat globules consisting of the lecithin-protein complex in addition to the casein and other substances usually adsorbed in ordinary mixes. This should leave the surface of the fat globules in such a condition that the serum will adhere more tenaciously and the mix should therefore whip better * * *. The fat globules represent a less serious point of weakness in the lamellae and therefore better whipping results.

[3] EICHBERG, J. Letter to senior author, Mar. 26, 1932.

Sommer's hypothesis seems logical, for the affinity of water being greater for proteins than for fat, the serum which composes the lamellae would adhere more readily to the fat globules if the outer layer of the oriented lecithin molecules was composed of protein.

Nitrogen determinations were made on the egg lecithin used in these experiments and an average of 0.079 percent of nitrogen was found in excess of that theoretically present in the accepted formula for lecithin. By using the factor 6.25 (*6*), this quantity of excess nitrogen is found to be equivalent to 0.5 percent of protein which could be present in combination with the lecithin as an adsorption film around the fat globule and improve the whipping properties, according to Sommer's hypothesis.

BUTTERMILK

Caulfield and Martin (*4*) attribute the failure of mixes containing butter to whip as readily as those containing cream to the churning process and not to the treatment the butter mixes receive. They found that the use of buttermilk in the mix resulted in only a slight improvement in the whipping properties. Tracy and Ruehe (*22*) also found that the longer time required for whipping in the case of butter mixes was not due to the absence of the buttermilk.

Whitaker (*24*) has obtained data which show that the addition of buttermilk to the butter mix does materially benefit the whipping properties and he suggests that the beneficial effect of the buttermilk is due to the lecithin that it contains. Martin (*9*) also has suggested that the lack of lecithin in butter mixes may have a bearing on their inferior whipping qualities. He states that the addition of powdered sweet-cream buttermilk has been found to be beneficial to the whipping properties.

The foregoing findings as to the effect of buttermilk in the butter mix, and the pronouncedly beneficial effect of egg lecithin noted in the present experiments, suggested the desirability of a further study of the effect of buttermilk on the whipping properties of butter mixes. Accordingly, fresh 40 percent cream was churned according to accepted methods. Some of the original cream, unsalted butter, and the buttermilk were used with dried skim milk and water to make ice-cream mixes. The results obtained are presented in table 6.

TABLE 6.—*Effect of buttermilk on the whipping properties of the mix and quality of the ice cream*

Type of mix	Time required to obtain 100 percent overrun	Score of ice cream after 3–5 days (average for 3 judges)			
		Flavor (perfect = 50)	Body and texture (perfect = 25)	Color (perfect = 5)	Total score (perfect = 80)
	Minutes				
Cream; dried skim milk; water	8.69	43.58	23.90	5	72.48
Butter; dried skim milk; water	12.00	43.26	23.90	5	72.10
Butter; dried skim milk; water; buttermilk * from above-mentioned cream	10.00	43.59	23.90	5	72.49

* Buttermilk supplied 38.5 percent of serum solids in 2 experiments and 1.6 percent in another experiment. The latter amount just equals the amount of serum solids supplied when cream is used as the source of fat.

Table 6 shows that the return of the buttermilk to the butter mix improved its whipping qualities. An average saving of 2 minutes to obtain the desired yield was effected by the addition of the buttermilk. The total score of the ice cream was slightly higher than that of the control mixes.

There has been considerable evidence that the phospholipids in milk are in combination with plasma protein (*7*, *14*, *15*, *16*, *17*), and Palmer and Wiese (*17*) have recently shown this to be true. Skim milk and butter contain only a very small amount of lecithin as compared with that contained in buttermilk (*1*). In view of the fact, then, that the lecithin is in combination with plasma proteins and that buttermilk contains the preponderance of the lecithin originally in the cream, and in view of the specific results obtained with egg lecithin and buttermilk in these experiments, the failure of butter mixes to whip as readily as cream mixes may be explained as due to a lack of the lecithin-protein complex. Sommer's (*21*) theory of the whipping phenomenon and his hypothesis regarding the lecithin-protein adsorption film around the fat globules are necessary adjuncts to this explanation.

SUMMARY AND CONCLUSIONS

The effects of various egg products, soybean lecithin, and buttermilk upon the whipping properties of ice-cream mixes and the quality of ice cream made from unsalted butter, dried skim milk, and water are presented.

The addition of 0.5 percent of dried egg yolk to butter-dried skim milk mixes greatly improved their whipping properties. The beneficial effect of egg yolk was more pronounced in mixes made with butter and dried skim milk than in mixes made with cream and dried skim milk.

The whipping properties of butter-dried skim milk mixes were improved equally by Chinese or domestic dried egg yolk. Dried whole egg improved whipping but did not give as good results as dried egg yolk. Sweetened frozen egg yolk materially improved the whipping properties of the mix but not to the same extent as dried egg yolk.

Of the various fractions separated from dried egg yolk, the vitellin was detrimental to the whipping properties. The egg-yolk salts exerted a slightly beneficial effect, as did also the portion of the yolk containing the salts and the proteins. The most pronounced beneficial effect was obtained with the portion of the yolk containing the lecithin. It is believed that certain of the egg-yolk proteins are in combination with the lecithin.

The addition of certain percentages of egg lecithin to butter-dried skim milk mixes gave decidedly beneficial results. Soybean lecithin, on the other hand, was detrimental to the whipping properties of the mix.

Sweet-cream buttermilk added to the butter-dried skim milk mix improved the whipping properties.

It is believed that the beneficial results of egg yolk on the whipping properties of butter mixes are due to a lecithin-protein complex in the yolk and that a similar lecithin-protein complex in buttermilk is responsible for its beneficial effect.

The data seem to indicate that the failure of butter mixes to whip as readily as cream mixes is due to the absence of the buttermilk and its lecithin-protein complex.

When dried skim milk and butter are used in making commercial ice-cream mixes, it is recommended that 0.5 percent of good quality dried egg yolk be used to improve the inferior whipping properties of such mixes.

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EFFECT OF METHOD OF SOWING ON THE YIELD AND ROOT AND TOP DEVELOPMENT OF SWEETCLOVER IN THE RED RIVER VALLEY [1]

By R. S. DUNHAM [2]

Agronomist, Northwest Experiment Station, University of Minnesota

INTRODUCTION

Sweetclover is a particularly important crop in the Red River Valley. It is especially adapted to the calcareous soils common to this area, it fits well into the average farm program and serves a variety of uses. It is commonly grown for pasture and seed production and less frequently for hay, but in connection with these uses it is generally believed to have value from the standpoint of soil improvement. In fact, its use primarily as a soil-improvement crop is increasing.

Extensive studies of the life history of sweetclover have been made in Ohio under humid conditions and a long growing season, and in Saskatchewan, a region of limited moisture supply and a short growing season. Climatic conditions in northwestern Minnesota are somewhat intermediate between these two.

It appeared desirable, therefore, to obtain definite information about the behavior of this crop in the Red River Valley, and a study was therefore made at the Northwest Experiment Station near Crookston, Minn. This paper presents data on yields of roots and tops harvested at various stages of growth as found in two types and two varieties of sweetclover sown with and without companion crops. It also presents some data on the nitrogen content of tops and roots.

REVIEW OF LITERATURE

It is generally agreed that where moisture is a limiting factor, the best stands of sweetclover are obtained when sown alone. However, except in very dry regions and on some light soils, seeding with companion crops is a general farm practice. As a result of investigations on the root development of sweetclover in Saskatchewan, Canada, where the precipitation during the growing seasons of 1928 and 1929 was 10.04 and 7.28 inches, respectively, Kirk [3] concluded:

> One could improve the soil as much in one season by growing sweetclover without a nurse crop as in 8 or 10 years by growing it with grain.

The competition of plants for survival and its influence on root development and top growth has been the subject of study by a few

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[3] KIRK, L. E. SWEET CLOVER AND SOIL IMPROVEMENT. Saskatchewan Univ. Col. Agr. Leaflet. 1931. [Mimeographed.]

investigators. Willard (*14*) [4] found that regardless of the initial stand, the final stand in mature sweetclover was about 30 plants per square yard. Piper (*10*) gives 7 plants per square foot or 63 per square yard as a perfect stand of mature sweetclover. McCool (*8*) in Michigan found striking differences between the root development of sweetclover plants thinned and unthinned.

Although the characteristics of the top growth and hay yields of the common sweetclover types and varieties are generally known, fewer investigations of the root growth and chemical composition have been made. Willard (*14*) found that biennial yellow produced a larger proportion and usually more roots than did the white sweetclover in the fall of the first year. Grundy County produced more roots and less tops than did the common white in the fall of the first year and less total yield the second year. Kirk compared several sweetclover varieties including white- and yellow-blossom types and found that they did not show any marked difference in the fertilizing value of their root material. Both Kirk (*7*) [5] and Willard (*14*) found there were no important differences in the composition of the tops of white and yellow sweetclover at corresponding stages. Arny and McGinnis (*2*) reported trials made in four sections of Minnesota. In all cases biennial white sweetclover outyielded biennial yellow in tops and roots in October of the first year. As an average of the 4 trials, the percentage of protein in the tops and the roots was approximately the same for the 2 types.

Hopkins in Illinois early recognized the promise of sweetclover as a green manure crop. He introduced it into the rotations on most of the 40 experimental fields throughout the State and selected it as the crop upon which to base his foundation work for restoring the soils of Greece. Bauer and De Turk, as reported by Keilholz (*6*), found from greenhouse studies with soil low in organic matter that—

> when sweet clover was grown on such soil the crop-producing power of it was improved, even tho both tops and roots were removed. The mere growing of the sweet clover crop helped the succeeding crop. The greatest benefit was secured, however, where the sweet clover was returned as a green manure.

Kirk [5] wondered—

> why sweet clover plowed down can increase the yields of corn 50 per cent in the corn belt, while its beneficial effects on the succeeding crop of grain can rarely be seen in the prairie plains sections of Western Canada.

The root development of plants has been found by various investigators (*9*, *11*, *12*, *13*) to depend on the type of soil, the water content of the soil, the depth of the water table, the species of plant, and the amount of top growth of the plant.

Aside from the development of roots as affected by various factors of environment, the study of root reserves also has received attention. Willard (*14*, *15*) and Kirk [5] in separate investigations have described the behavior of root reserves in sweetclover. Both investigators report a rapid decline in nitrogen and total dry matter as soon as growth begins in the spring of the second year.

The nitrogen content of types and varieties of sweetclover at various stages of growth and on different soils is of importance in a study of this plant as a green manure. Kirk (*7*, *p. 391*) found that the average

[4] Reference is made by number (italic) to Literature Cited, p. 994.
[5] KIRK, L. E. See footnote 3.

protein content does not differ significantly for the different sweetclover types, and Williams in Ohio (*16, p. 21*) reported that there were no important differences in the composition of white and yellow sweetclover at corresponding stages. Arny and McGinnis (*2*) found a maximum difference between biennial white and biennial yellow of 0.30 percent of nitrogen in the tops and 0.32 percent in the roots. Although the development of both tops and roots is affected materially by the type of soil (*2, 4, 8*), it seems possible that the total amount of nitrogen in an acre of sweetclover on different soils varies more because of a difference in yield than a difference in composition.

In comparisons of sweetclover at various stages of growth, Willard (*14*) found that the nitrogen content of the tops and roots in the fall of the first year did not vary greatly from 3 percent. In the early spring of the second year the roots contained from 4 to 4½ percent and in July about 1 to 1½ percent. Jones and Garrard (*5*) showed a decrease from 3.2 to 2.7 in the percentage of nitrogen in roots sampled April 26 and July 10, respectively. Kirk (*7*) found that the values for protein (in the tops) were almost identical in a fall cutting the first year, a first hay crop the second year, and a second hay crop the second year as an average of five "types" of sweetclover.

MATERIALS AND EXPERIMENTAL PROCEDURE

The surface soil of the plots used in this investigation is a very fine sandy loam, usually black to a depth of about 8 inches. The subsurface soil is a very fine sand and the subsoil a very calcareous lacustral clay. The depth to the clay varies from 12 to 40 inches. Hydrochloric acid will usually produce effervescence at the surface and always in the clay.

The field on which the plots were laid out had been cropped previously as follows: 1926, field peas; 1927, winter rye; 1928, barley; 1929, fallow; and 1930, potatoes.

Precipitation and temperature data are shown in table 1. The total precipitation for 1931 and until July 14, 1932, was less than average which under the drainage conditions of the station farm favored the experimental work. The mean temperatures were higher than the average.

TABLE 1.—*Precipitation and mean temperature at Crookston, Minn., during 1931–32 and 10-year average, 1920–29*

Item	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Precipitation:	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
1931	0.06	0.09	0.70	0.05	3.09	2.64	3.06	1.07	2.33	2.59	1.51	0.10	17.29
1932	.22	.54	.36	1.46	2.23	1.80	*1.01						
10-year average (1920–29)	.31	.52	.37	1.61	2.91	3.55	2.41	1.89	2.09	1.26	1.00	.36	18.28
Mean temperatures:	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
1931	17.0	25.6	27.4	44.6	53.2	67.8	70.9	67.6	63.5	49.5	32.4	19.1	44.9
1932	11.6	10.7	18.0	43.4	57.2	69.2	*71.6						
10-year average (1920–29)	5.4	10.6	24.5	41.5	55.0	62.9	68.8	66.7	58.0	44.9	26.2	10.7	39.6

* Until July 14 when last harvest of roots was made.

Two species or types of sweetclover, biennial white (*Melilotus alba*) and biennial yellow (*Melilotus officinalis*), and a variety of each, Grundy County and Albotrea, were used.

Biennial white is most commonly grown in the valley. It is a coarse rank-growing type used for the production of both seed and forage and for soil improvement.

Biennial yellow is finer stemmed and earlier than biennial white. Usually it yields less in hay. It is less desirable for pasture because of its early blossoming and its habit of producing seed close to the ground which shatters and reseeds the crop. It is less widely grown than the white but occurs throughout the valley largely because of mixture in seed of the common white.

Grundy County is a biennial dwarf white-blossom variety developed in Grundy County, Ill. It was at one time quite extensively used as a seed crop in the valley, but more recently it has largely lost favor among the farmers because of (1) a limited demand for the seed in this section, (2) its smaller yield of forage, and (3) the prevalent idea that it does not have so extensive a root system as either the biennial white or biennial yellow and therefore is of less value for soil improvement.

Albotrea is a yellow-blossomed biennial and probably a variety of the common yellow (*Melilotus officinalis*). The original plant was found growing along the roadside in Canada. At present Albotrea is of no commercial importance in the Red River Valley.

Each of the four kinds of sweetclover was sown in five ways (table 2): (1) With wheat (Ceres) as a companion crop, (2) with oats (Anthony) as a companion crop, (3) with barley (Trebi) as a companion crop, (4) without companion crop sown early (Apr. 29, 1931), and (5) without companion crop sown late (June 10, 1931). The companion crops were all planted April 29, 1931, at the usual rates of seeding; the sweetclover seed was sown at the rate of 12 pounds per acre.

Yields of both roots and tops were calculated on the basis of square-yard samples dried moisture free. Six square yards were taken from each plot for yields of tops and three for roots and stubble. The square-yard measures used are described by Arny and Steinmetz (*3*).

TABLE 2.—*Sweetclover plots and companion crops sown*

Sweetclover	Series 1					Series 2				
	Wheat	Oats	Barley	Alone early	Alone late	Wheat	Oats	Barley	Alone early	Alone late
Albotrea	1	2	3	4	5	6	7	8	9	10
Biennial yellow	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a
Grundy County	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b
Biennial white	1c	2c	3c	4c	5c	6c	7c	8c	9c	10c

In the first digging of roots made at the harvest of companion crops, the square yards were distributed over the plots at random. It was apparent, however, in these studies that this was not the best method of locating the areas to be dug. Since the stand of sweetclover was variable on the plots, it seemed best to group the square yards close together so that comparisons could be made between adjacent square-yard areas. Three areas of uniform stand on each

plot were selected and the square yards of the four subsequent diggings taken from these areas. The 3 square yards from each plot were bulked and treated as one sample. Figure 1 illustrates the arrangement and approximate location of the areas on the plots.

Harvests of tops and roots were made (1) at the time the companion crops were removed, (2) in October of the first year, (3) in May of the second year, (4) at the time of cutting the first hay crop, and (5) at the time of cutting the second hay crop.

In the first digging all roots were found in the upper 7 inches of the soil so that a 1-foot slice was amply deep. When the vertical roots tapered to a millimeter or less in diameter, no attempt was made to reach their extremities. Obviously, the yields of the second-year diggings are somewhat lower than they should be, but the findings of Willard (*14*), Arny (*2*), and Kirk[6] show this loss in weight to be small.

In addition to yields of tops and roots, the nitrogen content of the roots of Albotrea and biennial white and the tops of biennial white only was determined. In view of the fact that several investigators (*2, 15*)[6] have found no important difference in composition between sweetclover types, it was deemed inadvisable to extend the number of nitrogen determinations in this study.

In digging the roots it was impossible to recover all root nodules with the facilities available. To make the samples comparable, therefore, all nodules were stripped from the roots. Unquestionably this loss introduces an error in the nitrogen content of the roots, but it was unavoidable under the circumstances.

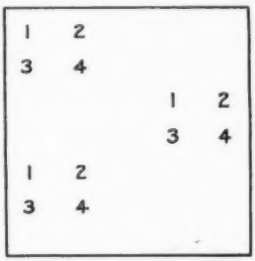

FIGURE 1.—Arrangement and location of square-yard areas on the plots. The times of harvest indicated are: 1, October 1931; 2, May 1932; 3, at time of cutting the first hay crop, June 10 to 14, 1932; 4, at time of cutting the second hay crop, July 14 to 19, 1932.

EXPERIMENTAL RESULTS

Yields of tops, roots, stubble, and the entire plant are reported in table 3. For ease of comparison, the table is discussed under these separate parts. Biometrical analyses of the data were made by Student's method (*1*) since any 2 adjacent square yards may be considered as paired comparisons. In the discussion of these comparisons, odds of 20 to 1 have been chosen arbitrarily as the minimum for indicating significant differences.

YIELDS OF TOPS

Wheat appears here to be better than oats or barley as a companion crop for each kind of sweetclover. Furthermore, no important difference in behavior is noticeable between the types and varieties when sown with either oats or barley. No harvests of sweetclover sown alone were made at the time of cutting the companion crops on the other plots nor was this sweetclover cut. Therefore the October yields of tops from the plots sown alone should not be compared with those from the sowings with companion crops, since the former had been allowed to grow all season whereas the latter had been cut with an 8-inch stubble in August.

[6] Kirk, L. E. See footnote 3.

TABLE 3.—*Average yield per acre of tops, roots, and stubble, and total dry weight (pounds) of 4 kinds of sweetclover when sown alone early and alone late, and with companion crops*

Method of sowing	Time of harvest	Albotrea				Biennial yellow				Grundy County				Biennial white				Average total weight, 4 kinds
		Tops	Roots	Stubble	Total	Tops	Roots	Stubble	Total	Tops	Roots	Stubble	Total	Tops	Roots	Stubble	Total	
Alone in April	October	1,771	3,549	409	5,729	1,823	2,941	338	5,102	1,007	2,134	304	3,445	2,232	2,532	430	5,194	4,867.5
	May	1,327	1,850	(a)	3,177	1,176	1,697	(a)	2,873	1,076	1,184	(a)	2,260	880	1,432	(a)	2,312	2,655.5
	First hay crop	1,087	1,829	1,430	4,346	1,255	1,573	1,550	4,378	1,364	1,332	1,636	4,332	1,459	1,595	1,740	4,794	4,462.5
	Second hay crop	1,564	1,437	820	3,821	1,656	1,753	978	4,387	1,746	1,261	1,012	4,019	2,028	1,744	934	4,706	4,233.2
Alone in June	October	957	2,012	207	3,176	868	1,927	192	2,987	375	863	86	1,324	743	1,289	157	2,189	2,419.0
	May	821	1,152	(a)	1,973	781	1,065	(a)	1,846	560	582	(a)	1,142	620	739	(a)	1,359	1,580.0
	First hay crop	797	1,103	1,117	3,017	904	913	1,195	3,012	1,116	613	1,210	2,939	1,258	859	1,213	3,330	3,074.5
	Second hay crop	1,717	952	569	3,238	1,755	714	661	3,130	1,896	563	720	3,179	2,311	836	684	3,831	3,344.5
With wheat	At grain harvest	804	318	508	1,630	536	122	269	927	204	51	138	393	334	89	266	689	909.7
	October, first year	306	1,742	247	2,295	265	1,531	233	2,029	183	1,081	191	1,455	281	1,222	203	1,706	1,871.2
	May, second year	1,097	1,065	(a)	2,162	1,080	997	(a)	2,077	763	649	(a)	1,412	970	807	(a)	1,777	1,857.0
	First hay crop, second year	1,334	1,027	1,254	3,615	1,540	1,090	1,291	3,921	1,694	837	1,246	3,777	1,834	978	1,116	3,928	3,810.2
	Second hay crop, second year	1,562	870	818	3,250	1,771	980	775	3,526	1,927	703	966	3,596	2,530	787	683	4,000	3,593.0
With oats	At grain harvest	330	85	186	601	160	45	157	362	81	33	136	250	145	34	129	308	380.2
	October	212	985	180	1,377	177	1,010	136	1,323	88	701	147	936	178	799	162	1,139	1,193.8
	May	615	646	(a)	1,261	699	626	(a)	1,325	471	508	(a)	979	507	511	(a)	1,018	1,145.8
	First hay crop	777	829	901	2,507	1,132	771	1,040	2,943	944	478	937	2,359	1,114	763	976	2,853	2,665.5
	Second hay crop	1,443	565	565	2,573	1,587	628	628	2,843	1,707	455	759	2,921	1,893	737	635	3,265	2,900.5
With barley	At grain harvest	319	172	286	777	209	70	218	497	105	42	141	288	261	62	232	555	529.2
	October	252	1,392	219	1,863	219	1,107	158	1,484	165	849	154	1,168	200	875	184	1,259	1,443.5
	May	704	857	(a)	1,561	732	691	(a)	1,423	648	665	(a)	1,313	673	594	(a)	1,267	1,391.0
	First hay crop	752	861	978	2,591	1,021	731	1,087	2,839	894	593	1,099	2,586	1,063	773	1,102	2,938	2,738.5
	Second hay crop	1,527	725	537	2,789	1,704	724	702	3,130	1,576	601	793	2,970	2,053	692	583	3,328	3,054.2

[a] Stubble not separated; included in weight of tops.

If the October yields are disregarded for these reasons, sowing with wheat likewise appears better than sowing alone in June for all types and varieties at all harvests except possibly in the case of the second hay crop of Albotrea. A comparison of the sowings with wheat and those sown alone in April reveals some variations between the sweetclovers. Biennial white yielded more in all 3 harvests from the sowing with wheat than from the plots sown alone, Grundy County and biennial yellow yielded more in 2 harvests, and Albotrea in only 1. An interesting observation is the fact that Albotrea, biennial yellow, and biennial white sown alone in April produced more top growth by October of the first year than in any later harvest.

No one kind of sweetclover yielded significantly better than any other at all harvests. At grain harvest, in October of the first year, and in May of the second year, Albotrea, biennial yellow, and biennial white had made larger top growths than Grundy County, although the odds that biennial white was superior in May are too low to be significant. As between Albotrea, biennial white, and biennial yellow at these harvests, there was no consistent superiority of one over the others. In the first hay crop both biennial yellow and Grundy County yielded more than Albotrea. Biennial white outyielded Albotrea and biennial yellow in both hay crops and also outyielded Grundy County in the second hay crop.

YIELDS OF ROOTS

The yields of roots given in table 3 are graphically illustrated in figure 2. Harvests from the plots sown alone were not made, so comparisons of sowing with and without a companion crop at this period are not possible.

In table 3 the yields of roots with wheat as the companion crop are larger for each kind of sweetclover at each harvest except one than with oats or barley. There appears to be no important difference between the sweetclovers when sown with barley or oats. In only 2 of the 32 harvests were the yields of roots larger from the sweetclover sown with oats than from that sown with barley. In all harvests of each kind of sweetclover, the yields of roots were larger from the sweetclovers sown alone in April than from the sowing with wheat. A comparison of the sweetclover sown alone in June with that sown with wheat shows no significant difference in yield of roots.

In a comparison of types and varieties in yields of roots, it is at once apparent that Grundy County made a root development inferior to the other kinds. Only at the time of the second hay crop, the odds that Albotrea yielded more are not significant. Both of the yellow-blossom types made a larger root development the first year than the biennial white. In the second year there is no significant difference between them. No one type or variety was superior to the other three throughout both seasons.

TOTAL WEIGHT OF PLANT

In the last column of yields in table 3 the combined weight of tops, roots, and stubble are reported as total weight. The weights of stubble are relatively high in the harvests at time of cutting the grain crop and at the first cutting of hay. At both times an 8-inch stubble was left because of cutting with a grain binder in the first instance and to avoid killing plants by too close cutting in the second case. The second crop of hay was cut with a 3-inch stubble.

On all plots where a companion crop was used and likewise where sown alone in June, the maximum total dry weight was produced generally at the time of cutting the second hay crop, but in 4 instances out of 16 at the time of cutting the first crop. Where sown alone in April, the maximum weight occurred in October of the first year among

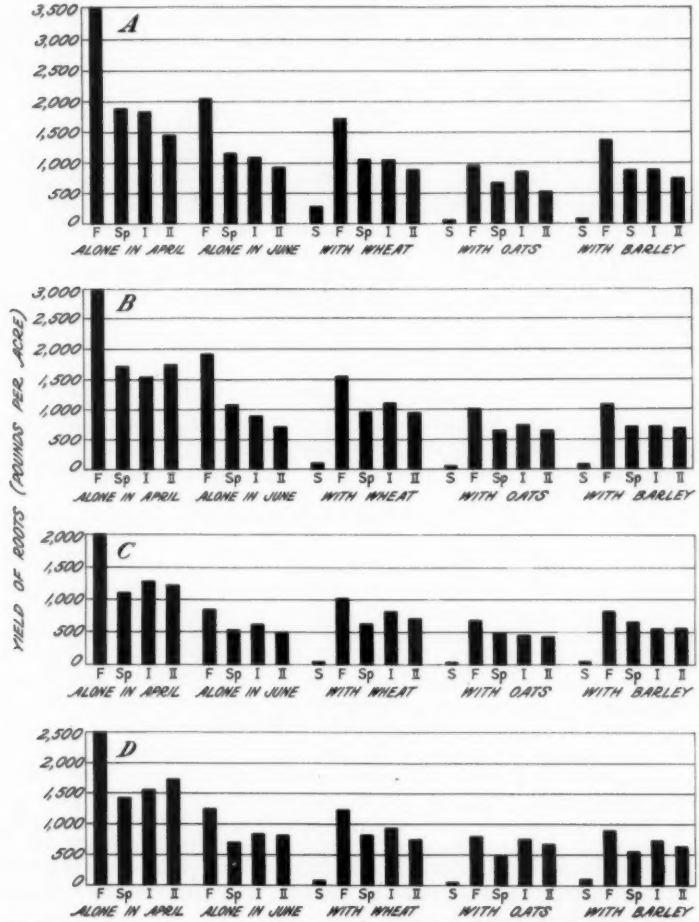

FIGURE 2.—Yields of Albotrea (*A*), biennial yellow (*B*), Grundy County (*C*), and biennial white (*D*), sweetclover roots at various periods, and when sown with and without companion crops: S=at grain harvest; F=October, first year; Sp=May, second year; I=first hay crop; II=second hay crop.

all types and varieties except Grundy County. From the standpoint of the entire plant, i.e., roots, stubble, and tops, the odds are not always significant that wheat was a better companion crop than oats or barley. However, the trend of the comparisons indicates this to be the case, since in no instance was sowing with either oats or barley superior to sowing with wheat. That sowing alone in April resulted

in larger total dry weights of the crop than sowing with wheat is definitely indicated. Sowing alone in June resulted in decidedly lower yields than sowing alone in April but was not significantly better or worse than sowing with wheat.

The data in table 3 show that the total dry weight of the first year was larger for the 2 yellow-blossom sweetclovers than for the 2 white. Subjected to Student's method (*1*) of comparison, the yellow-blossom sweetclovers yielded significantly more than the white except in the comparison of biennial white with biennial yellow. In only two comparisons were the odds significant throughout both seasons. Albotrea yielded significantly better than biennial white in the first 3 harvests and definitely less in the 2 hay crops. Biennial white yielded significantly greater during both seasons than Grundy County.

STAND IN RELATION TO METHODS OF SOWING

A determination of the stand of sweetclover following the five methods of sowing was made by counting the plants on 3 square yards of each plot and a like number on a duplicate plot. In table 4, comparisons of methods of sowing are shown with average differences and odds that the differences are significant. The comparisons are made using the four types and varieties of sweetclover. In a comparison of the desirability of the companion crops, only 3 counts out of 15 are significantly different. The number of plants in May, the second year, and at the time of the second hay crop when sown with wheat was greater than that sown with oats. The count of the sweetclover plants sown with barley was greater than where sown with oats at the time of the second hay crop. Sweetclover sown with wheat resulted in a larger stand in May of the second year than when sown alone in April and also larger than when sown alone in June.

TABLE 4.—*Comparison of the stands of 4 kinds of sweetclover when sown alone early and alone late and with companion crops*

Methods of sowing	Time of harvest	Average difference	Approximate odds
		Number of plants per square yard	
Barley *v.* oats	At grain harvest	2.8	−1.5:1
	October, first year	5.8	1.8:1
	May, second year	4.8	−1.5:1
	First hay crop, second year	−7.5	2.3:1
	Second hay crop, second year	42.2	47.8:1
Wheat *v.* sweetclover alone in April	October, first year	27.8	6.2:1
	May, second year	66.0	70.4:1
	First hay crop, second year	16.5	4.1:1
	Second hay crop, second year	10.3	2.3:1
Wheat *v.* barley	At grain harvest	21.0	4.1:1
	October, first year	21.8	4.1:1
	May, second year	21.4	2.8:1
	First hay crop, second year	7.1	1.8:1
	Second hay crop, second year	−6.5	1.8:1
Wheat *v.* oats	At grain harvest	23.6	6.2:1
	October, first year	27.5	7.8:1
	May, second year	69.1	59.2:1
	First hay crop, second year	−0.38	−1.5:1
	Second hay crop, second year	35.7	1,999:1
Wheat *v.* sweetclover alone in June	October, first year	23.8	5.2:1
	May, second year	45.8	26.8:1
	First hay crop, second year	19.1	7.8:1
	Second hay crop, second year	31.2	9.4:1

Since the yields of both roots and tops reported previously showed wheat to be definitely a better companion crop than oats or barley, it is apparent that yield was not dependent upon the number of plants per measured area in the first year.

The variation in stand at various periods and under different methods of sowing is shown in table 5. In all cases when sown with a companion crop, the stand increased from the time of grain harvest to October of the first year. With only two exceptions, the stand decreased in number of plants from October of the first year to July of the second year, at which time the second crop of hay was cut. The maximum decrease in stand from October or May till July was 51.48 percent, occurring when biennial white was sown with wheat. The average decrease in stand for all types and varieties was: With wheat, 34.77 percent; with oats, 36.61 percent; with barley, 25.49 percent; sown alone in April, 22.71 percent; and sown alone in June, 35.45 percent.

TABLE 5.—*Average number of sweetclover plants per square yard at successive periods for 4 kinds of sweetclover sown alone early and alone late and with companion crops*

Method of sowing and kind of sweetclover	At grain harvest	October, first year	May, second year	First hay crop	Second hay crop
With wheat:					
Albotrea	183	265	262	174	195
Biennial yellow	101	200	302	202	186
Grundy County	74	227	163	188	133
Biennial white	156	186	237	142	115
Average	128.5	219.5	241.0	176.5	157.2
With oats:					
Albotrea	107	255	201	235	146
Biennial yellow	92	200	191	185	147
Grundy County	118	150	163	147	94
Biennial white	107	160	136	141	98
Average	106.0	191.2	172.7	177.0	121.2
With barley:					
Albotrea	128	283	284	216	153
Biennial yellow	134	207	236	223	205
Grundy County	71	173	211	130	191
Biennial white	102	129	148	109	106
Average	108.7	198.0	219.7	169.5	163.7
Alone in April:					
Albotrea		164	187	172	161
Biennial yellow		235	159	147	128
Grundy County		170	179	160	173
Biennial white		192	176	162	126
Average		190.2	175.2	160.2	147.0
Alone in June:					
Albotrea		210	190	207	126
Biennial yellow		239	251	167	123
Grundy County		173	185	125	127
Biennial white		159	155	131	128
Average		195.2	195.2	157.5	126.0
Average of 4 kinds		198.8	200.8	168.2	143.0

ROOT DEVELOPMENT IN RELATION TO LIFE HISTORY OF THE PLANT

The dry weights of the roots per acre at various stages in the life history of the sweetclover plant and under different methods of sowing are reported in table 3 and graphically illustrated in figure 2. Of striking significance is the extremely small weight of the roots dug following the removal of the companion crops as reported in table 3 and illustrated in figure 2. Without a single exception among the four types and varieties or under any method of sowing, the maximum weight of roots was produced by October of the first year. The harvests in May were taken when the sweetclovers had made from 4 to 6 inches top growth. In every case the weight of roots was less than in the

preceding fall. Generally there was some increase in the weight of roots from May till the time of cutting the first hay crop, and in no case was there a large decrease. In only one half of the trials did the weights of roots from the last digging represent the minimum for all harvests subsequent to that at grain harvest.

TABLE 6.—*Average weight (grams) at successive periods of roots from single plants of 4 kinds of sweetclover sown alone early and alone late, and with companion crops*

Method of sowing and time of harvest	Albotrea			Binenial yellow			Grundy County			Biennial white			Average of 4 kinds
	Plot 4	Plot 9	Average	Plot 4a	Plot 9a	Average	Plot 4b	Plot 9b	Average	Plot 4c	Plot 9c	Average	
Sown alone in April:													
October, first year	1.91	2.15	2.03	1.12	1.21	1.16	1.23	1.14	1.18	1.28	1.14	1.21	1.40
May, second year	1.29	.73	1.01	1.02	.98	1.00	.66	.59	.62	.81	.74	.78	.85
First crop, second year	.85	1.33	1.09	1.13	.93	1.03	.65	.96	.80	.75	1.06	.90	.96
Second crop, second year	.82	.84	.83	1.29	1.26	1.28	.97	.53	.75	1.37	1.24	1.30	1.04
	Plot 5	Plot 10	Average	Plot 5a	Plot 10a	Average	Plot 5b	Plot 10b	Average	Plot 5c	Plot 10c	Average	
Sown alone in June:													
October, first year	0.63	1.14	0.88	0.73	0.77	0.75	0.31	0.60	0.46	0.41	1.11	0.76	0.71
May, second year	.38	.76	.57	.44	.36	.40	.19	.38	.28	.35	.54	.44	.42
First crop, second year	.40	.60	.50	.49	.52	.50	.42	.50	.46	.49	.70	.60	.52
Second crop, second year	.53	.86	.70	.63	.47	.55	.40	.43	.42	.54	.69	.62	.57
	Plot 1	Plot 6	Average	Plot 1a	Plot 6a	Average	Plot 1b	Plot 6b	Average	Plot 1c	Plot 6c	Average	
Sown with wheat:													
Grain harvest	0.31	0.10	0.20	0.30	0.08	0.19	0.027	0.068	0.048	0.052	0.054	0.053	0.12
October, first year	.60	.65	.62	.85	.59	.72	.49	.42	.46	.82	.48	.65	.61
May, second year	.44	.32	.38	.37	.25	.31	.45	.32	.38	.46	.24	.35	.36
First crop, second year	.56	.55	.56	.64	.40	.52	.43	.41	.42	.76	.54	.65	.54
Second crop, second year	.39	.46	.42	.85	.28	.56	.77	.36	.56	.72	.58	.65	.55
	Plot 2	Plot 7	Average	Plot 2a	Plot 7a	Average	Plot 2b	Plot 7b	Average	Plot 2c	Plot 7c	Average	
Sown with oats:													
Grain harvest	0.13	0.048	0.089	0.071	0.030	0.050	0.027	0.026	0.026	0.024	0.031	0.028	0.05
October, first year	.34	.38	.36	.62	.36	.49	.50	.38	.44	.42	.52	.47	.44
May, second year	.33	.28	.30	.50	.23	.36	.34	.24	.29	.37	.34	.36	.33
First crop, second year	.35	.30	.32	.68	.24	.46	.34	.28	.31	.65	.42	.54	.41
Second crop, second year	.31	.48	.40	.54	.32	.43	.63	.35	.49	.81	.63	.72	.51
	Plot 3	Plot 8	Average	Plot 3a	Plot 8a	Average	Plot 3b	Plot 8b	Average	Plot 3c	Plot 8c	Average	
Sown with barley:													
Grain harvest	0.16	0.10	0.13	0.081	0.038	0.060	0.063	0.048	0.056	0.080	0.036	0.058	0.08
October, first year	.47	.46	.46	.56	.46	.51	.40	.54	.47	.66	.62	.64	.52
May, second year	.30	.27	.28	.31	.24	.28	.28	.32	.30	.35	.41	.38	.31
First crop, second year	.33	.44	.38	.39	.26	.32	.37	.50	.44	.81	.58	.70	.46
Second crop, second year	.41	.48	.44	.36	.31	.34	.29	.30	.30	.72	.52	.62	.42

A question of considerable interest is whether the individual roots increase or decrease in weight the second year. A loss in the total weight of roots per acre does not necessarily indicate a loss in weight of the individual roots since such total loss might be caused by a decrease in stand due to natural competition of the plants. Furthermore, as Willard (*14, p. 38*) states:

* * * the plants that die weigh much less than those that remain alive and the latter would therefore apparently increase in weight whether they actually grew or not.

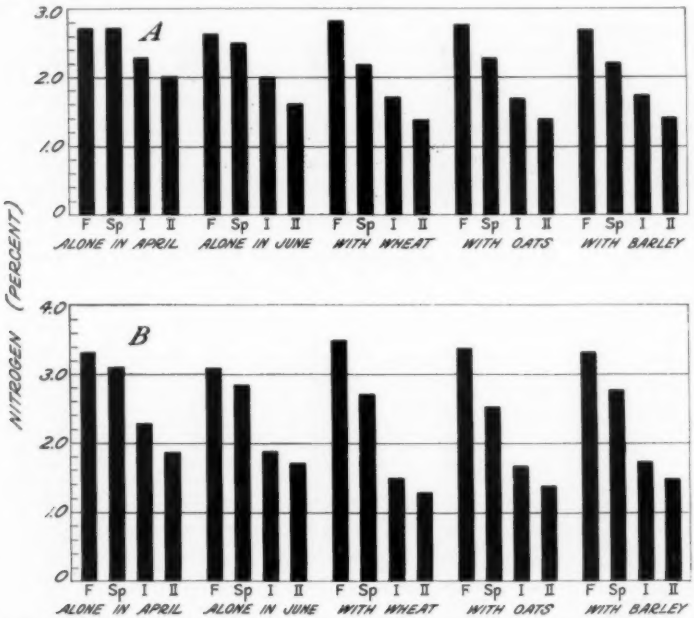

FIGURE 3.—Percentage of nitrogen in the roots of Albotrea (*A*) and biennial white (*B*) sweetclover at various periods and when sown with and without companion crops: F, October, first year; Sp, May second year; I, first hay crop; II, second hay crop.

The average weights of roots from single plants are given in table 6. These were obtained by dividing the total weights of roots per square yard by the number of plants. Of 40 such samples, 25 showed a decrease in weight of individual roots from October of the first year to July of the second year, 14 showed an increase, and 1 was the same. In 5 of the 25 samples showing a decrease in weight, the number of plants in July was greater than in October so that such apparent decrease could have resulted from the increased number. By the method of dividing the total weight by the number of plants an increased number would naturally tend to show a decrease in the weights of the individual roots. In all other samples there was a lower number of plants in July than in October of the first year.

PERCENTAGE OF NITROGEN IN TOPS AND ROOTS

The percentage of nitrogen found in the tops of the biennial white sweetclover at the first and second cuttings of hay and the percentage of nitrogen in the roots of biennial white and Albotrea at four stages

of growth are presented in table 7. Very slight variations occurred in the nitrogen content under different methods of sowing. The amount contained in the second cutting, however, was definitely lower than in the first.

In figure 3, the nitrogen content of the roots of Albotrea and biennial white is illustrated at 4 harvests and under 5 methods of sowing. The decrease in nitrogen content from October of the first year to the second cutting of hay is pronounced and fairly uniform for all methods of sowing. In table 7, the acre yields of nitrogen have been computed. Although the percentage of nitrogen did not vary greatly between different methods of sowing, the acre yield of nitrogen shows important differences.

TABLE 7.—*Percentage of nitrogen and pounds of nitrogen per acre in tops and roots of biennial white and in roots of Albotrea at 4 stages of growth, when sown alone early and alone late, and with companion crops*

PERCENTAGE OF NITROGEN

Method of sowing	Biennial white tops		Biennial white roots				Albotrea roots			
	First hay crop	Second hay crop	October, first year	May, second year	First hay crop	Second hay crop	October, first year	May, second year	First hay crop	Second hay crop
Alone in April	3.12	2.22	3.30	3.11	2.24	1.87	2.71	2.69	2.25	1.99
Alone in June	3.20	2.13	3.06	2.82	1.86	1.71	2.61	2.53	1.97	1.61
With wheat	3.11	2.21	3.44	2.68	1.56	1.30	2.80	2.18	1.66	1.39
With oats	3.27	2.36	3.37	2.57	1.66	1.39	2.76	2.26	1.69	1.40
With barley	3.12	2.26	3.26	2.74	1.71	1.52	2.64	2.21	1.68	1.40
Average	3.16	2.24	3.29	2.78	1.81	1.56	2.70	2.37	1.85	1.56

POUNDS OF NITROGEN PER ACRE

Alone in April	45.5	45.0	83.6	44.5	35.7	32.6	96.2	49.8	41.2	28.6
Alone in June	40.3	49.2	39.4	20.8	16.0	14.3	52.5	29.2	21.7	15.3
With wheat	57.0	55.9	42.0	21.6	15.3	10.2	48.8	23.2	17.0	12.1
With oats	36.4	44.7	26.9	13.1	12.7	10.2	27.2	14.6	14.0	7.9
With barley	33.2	46.4	28.5	16.3	13.2	10.5	36.8	18.9	14.5	10.2
Average	42.5	48.2	44.1	23.3	18.6	15.6	52.3	27.1	21.7	14.8

DISCUSSION

This investigation was conducted under favorable conditions. The soil contained sufficient lime for successful growth of sweetclover. It represented a type intermediate between the heavy and light soils of the valley and was in a good state of fertility. The seasons of 1931 and 1932 were drier and warmer than the 10-year average, but under the drainage conditions of the experimental farm such seasons are more favorable than abnormally wet years.

Ceres wheat, Anthony oats, and Trebi barley, high-yielding varieties extensively used in the valley, were chosen for the companion crops. There was no lodging of any grain crop so this factor did not enter into the comparison. However, the apparent definite superiority of wheat as a companion crop for sweetclover may have been due to the variety of oats used. It is possible that an early, short-growing oat would have proved better as a companion crop than Anthony which is mid-season and mid-tall. On the other hand, farmers select grain crop varieties primarily for yield and only secondarily as companion crops.

The June seeding was handicapped by weeds which generally prevail in sowing without companion crops, although the ground was kept cultivated until seeding time. The early seeding grew fast enough

to keep the weeds in better control. For root development there appears to be a decided advantage in sowing alone early. For maximum yield of roots such seeding is definitely superior to the use of a companion crop not only during the first year but continuing throughout the second season. The practicability of sowing alone, however, is open to question, since the loss of the grain crop is entailed and weeds are not kept so well under control.

It is evident from the data that some sweetclover seeds sown with the grain crops did not germinate until after the companion crop was removed since in every instance there were more plants per square yard in October of the first year than at the time of grain harvest. Apparently the stands obtained by all methods of sowing were adequate under the favorable conditions of the trial, since no significant relationship existed between stand the first year and subsequent yields. Moreover, the ultimate stand was in all cases much larger than that found by Willard (*14*). An analysis of 15 comparisons of stand showed (1) that in only 3 instances a thicker stand resulted in a better yield of roots and tops, (2) that in no instance did a decrease in stand result in either a better or poorer yield, and (3) that in 12 instances there was no significant relationship of any kind.

In yields of hay, the biennial white ranked first, Albotrea last, and the biennial yellow and Grundy County about equal and in an intermediate position. Choice of variety for a hay crop, however, might depend on other factors than yield.

Whether the individual roots increase or decrease in weight from late fall of the first year to July of the second year is not clear, although the data appear to show a decrease. The possibilities of drawing conclusions from the data obtained may be stated as follows:

(1) If an increase in stand is accompanied by a loss in weight of the individual roots, no conclusion concerning this question is justified since the total weight of the roots per square yard divided by an increased number would tend to show a loss in weight.

(2) If loss in stand is accompanied by an increase in weight, no conclusion is justified since the plants that died were presumably of less weight than those that lived and hence would tend to show an increase in weight of the latter.

(3) If an increase in stand is accompanied by an increase in weight, the conclusion is justified that the individual roots increased in weight.

(4) If a loss in stand is accompanied by a loss in weight, the conclusion is justified that the individual roots actually lost in weight.

An examination of 40 comparisons using these possibilities of conclusions showed a loss of weight in 20 and no justifiable conclusion in the remaining 20.

Grundy County proved inferior to the other kinds of sweetclover in root development. For plowing under in the fall of the first year or early spring of the second year, the two yellow kinds were superior to the white since they made both a significantly larger root development and in 3 comparisons out of 4 a significantly larger total weight of tops, stubble, and roots than the white. In the second year, however, this did not hold true of the total weight, and root production was about the same. Albotrea was superior to the biennial yellow as a crop for plowing under in the fall of the first year or early spring of the second year, and biennial white was definitely better than Grundy County for plowing under in either year.

The maximum root development and nitrogen content occurred without exception in the late fall of the first year. The maximum total weight occurred at the time of the first or second hay crop when sown with a companion crop or alone in June. When the sweetclover was sown alone in April, this occurred in October of the first year, with the exception of Grundy County. No nitrogen determinations were made for tops in the fall. If it is assumed that the percentage of nitrogen is the same as in the first hay crop, as Kirk (7) found, the maximum total nitrogen per acre in both tops and roots of biennial white occurred in October of the first year.

As far as total weight and nitrogen per acre in this investigation are concerned, the best time to plow under sweetclover for soil improvement is in the late fall of the first year and the best method of sowing is early without a companion crop. However, other factors not investigated in these trials are important. It is very difficult to kill sweetclover in the fall of the first year. Plowing under the maximum amount of organic matter may not prove most successful for succeeding crops. Weeds may make sowing sweetclover alone impracticable even though seeded early, and the loss of the grain crop is an economic factor. The next best method, again considering total weight and nitrogen per acre, is to sow with wheat and plow under either the first or second crop. Again other factors not investigated are present. Plowing under the first crop leaves too short a season in the valley for planting most other crops and a long season for fallowing. Furthermore, plowing under the maximum amount of organic matter may not prove the best method. Investigations in the methods of handling biennial white sweetclover for soil improvement under the supervision of the soils division, University Farm, are now under way at Crookston.

SUMMARY

Two types of sweetclover, biennial white (*Melilotus alba*), biennial yellow (*Melilotus officinalis*), and a variety of each, Grundy County and Albotrea, were grown at the Northwest Experiment Station, Crookston, Minn., in 1931 and 1932. A comparison was made of yield of roots and tops of the four types at five stages of growth, sown with and without companion crops. The nitrogen content of tops and roots of biennial white and Albotrea was studied.

Larger yields of tops were obtained from all four kinds of sweetclover when sown with Ceres wheat, than with Anthony oats or Trebi barley. With the exception of Grundy County, all sweetclovers sown alone in April produced more top growth by October of the first year than in any harvest the second year under the same method of sowing. Grundy County, however, yielded most in the second hay crop the second year.

The maximum yield of roots was produced by each sweetclover when sown alone in April. When sown with companion crops, the largest yield of roots was produced from the sweetclover sown with wheat, and the least from that sown with oats.

When the sweetclovers were sown alone in April the maximum total weight of roots, tops, and stubble occurred in October of the first year for all except Grundy County. The latter made its maximum development at the time of cutting the first hay crop of the second year.

When sown with a grain crop or alone in June, however, the maximum total weight was produced in the second year, 12 times at the second hay crop and 4 times at the first.

Stands were not greatly affected by the type of companion crop used. The stand increased when sown with companion crops from the time the grain was removed until October of the first year. In every comparison but two the stand decreased from October of the first year to July of the second. The average decrease for all sweetclovers was: 34.77 percent sown with wheat; 36.61 percent with oats; 25.49 percent with barley; 22.71 percent sown alone in April; and 35.45 percent sown alone in June.

Grundy County gave a lower yield of roots than did the other three varieties. Differences in yield of tops for the other three varieties were not significant.

In the first year the two yellow sweetclovers produced larger total yields of roots, stubble, and tops than the white in 3 out of 4 comparisons, but this did not hold true for the second year. When sown alone in April, Albotrea and biennial white produced the largest total weights of all types and varieties in October of the first year.

Without any exception, the maximum weight of roots was found in October of the first year. In every instance the weight of roots was less the following May. Individual roots appear to decrease in weight from October of the first year to July of the second season.

The nitrogen percentage in the tops of biennial white was lower in the second hay crop than in the first and was not greatly influenced by the different methods of sowing. The nitrogen percentage in the roots was much lower in July of the second season than in October of the first year.

The maximum total nitrogen per acre in both tops and roots of biennial white was produced in October of the first year.

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TEMPERATURE RELATIONS OF WOOD-DESTROYING FUNGI [1]

By C. J. HUMPHREY, formerly *pathologist*, and P. V. SIGGERS, *associate pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

A knowledge of the rate of growth of wood-destroying fungi at different temperatures is of considerable importance to the pathologist working on problems of timber decay. In fact, the effectiveness of control measures may at times be largely dependent upon the behavior of the organism under the temperature conditions imposed. This is particularly true of certain dry-rot fungi whose development may be arrested by raising, as far as practicable, the temperature of the air in buildings.

The present paper records the growth, on malt-extract agars in Petri dishes, of 56 named species and several strains of fungi, most of which are commonly found in various parts of the United States and Cuba, and some of which are cosmopolitan in their distribution. A considerable number that are of great economic importance are represented on account of the severe decay which they cause in structural timber.

MATERIALS AND METHODS

Two different media were used, each of which contained malt extract. Medium 1 had the following composition: Distilled water, 1,000 cc; beef extract (Armour's), 5 g; Löfflund's malt extract, 25 g; agar-agar, in strips, 20 g. The mixture was carefully filtered, but the reaction was not adjusted and remained somewhat acid. This is a very good medium for the development of wood-destroying fungi.

Medium 2 consisted of distilled water, 1,000 cc; Trommer's malt extract (plain), 25 g; agar-agar (Bacto, granulated), 20 g. The mixture was filtered, but the reaction was not adjusted (+8.5 to +10.5, Fuller's scale).

Seven liters of medium 1 was prepared at one time. The agar was dissolved in several 2-l Erlenmeyer flasks by adding the desired amount of water and cooking in an autoclave at 100° C. for about 1¼ hours. In order to make the medium as uniform as possible the different lots were then mixed in an enamel-ware pan. To the pan of hot solution were added the necessary amounts of beef extract and malt extract, which were then thoroughly mixed with the agar. The medium was then filtered through cotton, tubed in 20-cc quantities, and sterilized in the autoclave for 40 minutes at a pressure of 15 pounds.

Eighteen liters of medium 2 was made at one time. This was done by placing the Bacto agar in 2-l Erlenmeyer flasks containing 1,500 cc of distilled water and heating for 2½ hours in the autoclave at 100°

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[2] The writers wish to express their appreciation to Mrs. Ruth M. Fleming for much assistance during the early part of the investigations reported herein.

C. The separate quantities were then poured into a large enamelware pan, which was kept warm by a Bunsen burner until the malt extract had been added, and the medium was then filtered through cotton into 1,000-cc Erlenmeyer flasks. Without allowing the agar to harden, the flasks were sterilized in the autoclave for 30 minutes without pressure. On each of the two following days they were again heated for 1½ hours without pressure. After cooling they were placed in the ice box for use as needed.

Whenever tests were started the necessary number of flasks were steamed for an hour at 100° C., and then the contents were tubed in 20-cc quantities and sterilized for 30 minutes without pressure on each of 3 successive days. On the third day the contents of the tubes were poured while hot into sterile Petri dishes.

The Petri dishes used in the tests were 100 mm in diameter and 12 to 15 mm deep. The lids were carefully selected so as to secure a close fit and avoid unnecessary drying of the medium. After it was poured, the agar was inoculated at the center of the plates by depositing a square of fungus growth, usually 8 by 10 mm in size, but occasionally smaller, that had been cut from near the margin of another Petri-dish culture and was therefore in a vigorous growing condition.

The cultures from which these squares were taken differed somewhat in age. Those used for medium 1 (first series) usually ranged from 13 to 22 days and were rarely 60 days old; those used for medium 2 (second series) ranged from 11 to 25 days and were occasionally 31 days old. As far as could be determined, however, the difference in age had no apparent influence on the rate of growth. This was probably due to the fact that the squares of inoculum were cut from the growing margin or near it.

After inoculation, the dishes were stacked in electric or gas-heated incubators maintained at the desired temperature by thermostatic control. For temperatures above that of the air in the room a water-jacketed electric incubator was usually employed; for lower temperatures a gas-heated Hearson incubator was used. The variation of temperature in these incubators at the points where the thermometers were inserted was usually about 0.5° C. The variation was probably greater between widely separated points in the chamber, because the heavy loading impeded air circulation and the application of heat at the bottom of the electric incubators tended to raise the temperature at that point above the desired limit. However, crowding of the cultures ceased as a factor in temperature variation from 38° to 46°, since at these temperatures heavy loading never occurred. The Hearson incubators were more uniform in temperature within, since in these the temperature is controlled automatically by adding small quantities of hot or cold water to the jacket as needed.

The thermometers used were checked against a standardized instrument. During tests at the different temperatures in the second series (on medium 2) the averages of all the readings recorded in no case varied more than 0.6° C. from the desired temperatures.

Radial growth in millimeters from the edge of the inoculum was recorded at the end of the first and second weeks. Measurements were made with a thin millimeter scale held against the inverted dish. Since growth was not always uniform on all sides of the inoculum, the average was computed as nearly as possible.

In the first series (on medium 1) the plates were made in duplicate, and the value given for rate of growth represents the average for the two plates. In the second series (on medium 2) the cultures were prepared and averaged in triplicate.

An alphabetical tabulation of species and cultures studied, together with their sources and an index of the graphs, is given in table 1. The stock cultures used were obtained by the senior writer and were mostly his own isolations.

TABLE 1.—*List of species and cultures with their sources and localities, and an index of the graphs*

Fungus	Source	Locality	Fig. no.
Collybia velutipes (Curt.) Fr.	Spores	Wisconsin	1, *C*
Coniophora cerebella Pers., no. 1		Europe [a]	1, *D*
Coniophora cerebella Pers., no. 2	Spores	Illinois	2, *O*
Corticium chrysocreas Berk. and Curt	do	Louisiana	2, *G*
Corticium effuscatum Cke. and Ell	do	Illinois	2, *L*
Daedalea ambigua Berk	do	Mississippi	2, *Q*
Daedalea quercina (L.) Fr.	Sporophores	Massachusetts	3, *K*
Daedalea unicolor (Bull.) Fr.	Spores	Wisconsin	2, *P*
Fomes annosus (Fr.) Cke., no. 1		Europe [a]	1, *E*
Fomes annosus (Fr.) Cke., no. 2	Sporophores	Pennsylvania	3, *L*
Fomes everhartii Ell. and Gall	do	Wisconsin	2, *R*
Fomes igniarius (L.) Gill	do	Montana	3, *E*
Fomes marmoratus (Berk. and Curt.) Cke	do	Florida	2, *S*
Fomes nigrolimitatus Romell	do	Washington	1, *F*
Fomes officinalis Fr.	do	do	1, *J*
Fomes pinicola (Swendener) Cke	do	Montana	1, *V*
Fomes rimosus Berk	do	Ohio	2, *T*
Fomes subroseus Weir	do	United States	2, *E*
Ganoderma applanatum (Pers.) Pat	do	Indiana	1, *I*
Ganoderma lucidum (Leys.) Karst	do	Wisconsin	3, *R*
Hydnum ochraceum (Pers.) Fr	Spores	do	1, *N*
Hydnum pulcherrimum Berk. and Curt	do	Louisiana	3, *G*
Irpex mollis Berk. and Curt	do	Wisconsin	3, *H*
Lentinus lepideus Fr., no. 1	do	Montana	2, *C*
Lentinus lepideus Fr., no. 2	do	Wisconsin	3, *B*
Lenzites berkeleyi Lév	Sporophores	Florida	3, *M*
Lenzites saepiaria (Wulf.) Fr.	Spores	Wisconsin	2, *F*
Lenzites striata (Sw.) Fr.	do	Cuba	3, *N*
Lenzites trabea (Pers.) Fr., no. 1	do	Wisconsin	3, *O*
Lenzites trabea (Pers.) Fr., no. 2	do	Illinois	3, *P*
Merulius himantioides Fr.	do	Wisconsin	2, *A*
Merulius lacrymans (Wulf.) Fr., no. 1		Europe [a]	1, *B*
Merulius lacrymans (Wulf.) Fr., no. 2	Building timber	Rhode Island	1, *A*
Merulius silvester Falck [b]		Europe [a]	1, *K*
Merulius tremellosus (Schrad.) Fr.	Spores	Florida	3, *I*
Panus rudis Fr.	do	Minnesota	3, *S*
Peniophora gigantea (Fr.) Mass.	do	Michigan	1, *T*
Phlebia merismoides Fr.	do	do	1, *Q*
Phlebia strigoso-zonata (Schw.) Lloyd	Decayed wood	Minnesota	2, *V*
Pleurotus ostreatus (Jacq.) Fr.	Spores	Wisconsin	2, *K*
Polyporus maximus (Mont.) Overh.	do	Cuba	2, *U*
Polyporus radiatus (Sow.) Fr.	Sporophores	Wisconsin	1, *L*
Polyporus robiniophilus (Murr.) Lloyd	do	Ohio	2, *J*
Polyporus schweinitzii Fr.	do	Florida	1, *M*
Polyporus sulphureus (Bull.) Fr.	do	Montana	2, *D*
Polystictus abietinus (Dicks.) Fr. (*Irpex fusco-violaceus* (Schrad.) Fr.).	Spores	New York	1, *O*
Polystictus abietinus (Dicks.) Fr.	Decayed wood	Wisconsin	2, *H*
Polystictus hirsutus (Wulf.) Fr.	Spores (?)	do	3, *C*
Polystictus sinuosus Fr.	Spores	New York	3, *I*
Polystictus tenuis (Lk.) Cke.	do	Cuba	3, *A*
Polystictus versicolor (L.) Fr.	Sporophores	Wisconsin	2, *I*
Poria incrassata (Berk. and Curt.) Burt	Building timber	Mississippi	1, *R*
Poria subacida (Pk.) Sacc.	Spores	Michigan	1, *P*
Poria xantha Fr. sensu Lind.	do	Montana	1, *S*
Schizophyllum commune Fr., no. 1	do	Wisconsin	3, *C*
Schizophyllum commune Fr., no. 2	do	Cuba	3, *D*
Stereum fasciatum Schw.	do	Wisconsin	2, *N*
Stereum frustulosum (Pers.) Fr.	do	do	1, *U*
Stereum fuscum (Schrad.) Quel.	do	Florida	3, *J*
Stereum gausapatum Fr.	do	Wisconsin	2, *M*
Stereum rameale Schw.	do	do	1, *W*
Trametes pini (Thore) Fr.	Decayed wood	Idaho	1, *H*
Trametes serialis Fr., no. 1	Sporophores	Wisconsin	2, *B*
Trametes serialis Fr., no. 2	Spores	do	1, *G*

[a] Culture from Dr. Westerdijk, Netherlands.

[b] *Merulius silvester* is considered synonymous with *M. himantioides*.

EXPERIMENTAL DATA

In order to present the growth data in a form easily comprehensible, graphs (figs. 1–3) have been prepared for each of the 64 species or strains studied. The general arrangement of the graphs is in the order of increasing maximum temperatures at which growth ceases;

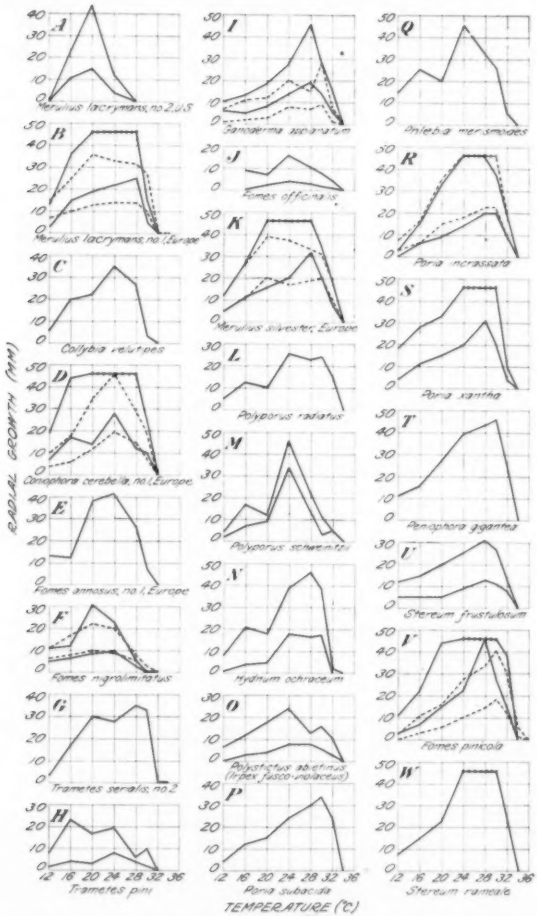

FIGURE 1.—Graphs (*A–W*) showing growth of 23 wood-destroying fungi at different temperatures. A solid line represents 1 week's growth on medium 1; a broken line represents 1 week's growth on medium 2. Where two solid lines or two broken lines are shown, the upper represents 2 weeks' growth. A dotted line indicates that the fungus grew to the edge of the Petri dish before the first measurement was made.

thus the graphs with the lowest maxima are grouped on figure 1, and those with the highest maxima appear on figure 3. Since the rate of growth during the second week may present a smoother curve than that during the first week, on account of the equalization of slight irregularities that occurred in the development of the fungus

during the first 2 or 3 days while it was becoming established, the data for both 1 and 2 weeks are plotted wherever available. The data for the second week, however, would have been of no significance in the case of certain species which grew so rapidly that they covered the

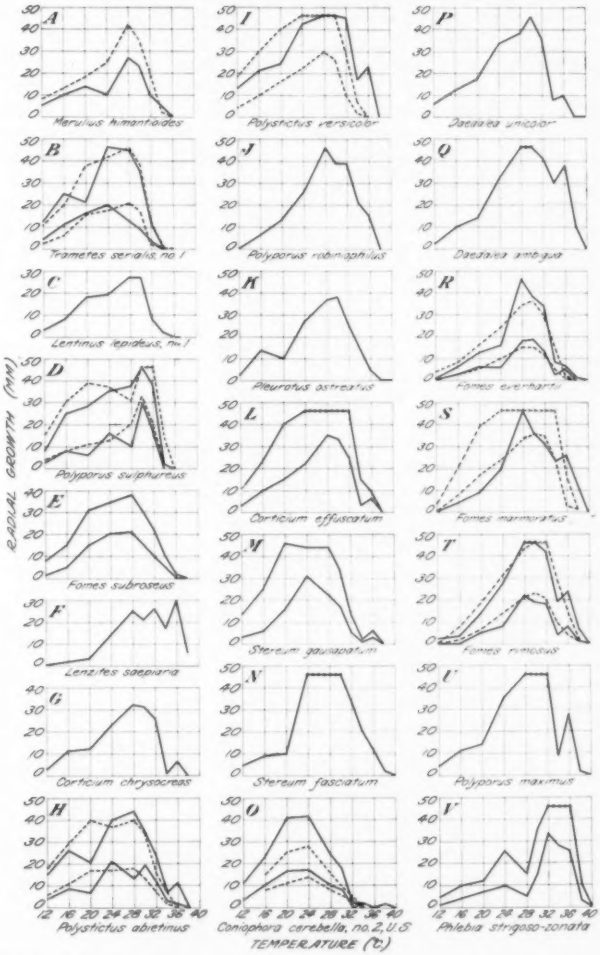

FIGURE 2.—Graphs (*A*-*V*) showing growth of 22 wood-destroying fungi at different temperatures. A solid line represents 1 week's growth on medium 1; a broken line represents 1 week's growth on medium 2. Where two solid lines or two broken lines are shown, the upper represents 2 weeks' growth. A dotted line indicates that the fungus grew to the edge of the Petri dish before the first measurement was made.

plates during the first week over a considerable temperature range. Had larger dishes been used or had measurements been taken at shorter intervals, the rate of growth could have been more carefully checked and the optimum temperature for the rapidly growing species could, in many instances, have been more closely defined.

In some of the later work with medium 2, an attempt was made to provide a larger radius for growth by planting the inoculum on one side of the dish in the case of rapidly developing species. In other instances the short period required to cover the dish was recorded

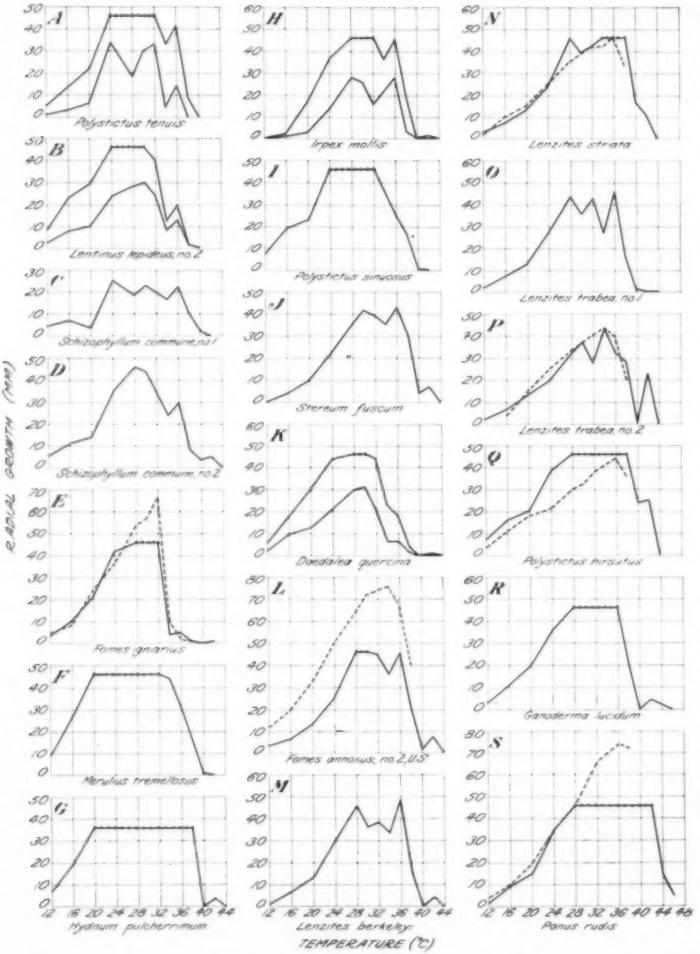

FIGURE 3.—Graphs (*A*–*S*) showing growth of 19 wood-destroying fungi at different temperatures. A solid line represents 1 week's growth on medium 1; a broken line represents 1 week's growth on medium 2. Where two solid lines or two broken lines are shown, the upper represents 2 weeks' growth. A dotted line indicates that the fungus grew to the edge of the Petri dish before the first measurement was made.

and the total growth for the week was computed on the assumption that the rate of growth was constant. There are possible errors in both methods. An inhibition of growth on one side of the inoculum, due to proximity to the side of the dish, may possibly result in stronger

growth on the free side, and it cannot safely be assumed in either case that a uniform rate of growth is maintained from the beginning. In fact, as a number of the graphs indicate, acceleration in growth occurred during the second week of observation. Monteith and Dahl,[3] working with nine strains of *Rhizoctonia solani* Kuehn, also found a difference in the rapidity with which new cultures started growth, even when transfers were taken from corresponding segments of mycelium from the same plate; however, when once established they grew at nearly the same rate.

While the plotted data indicate in most cases a fairly regular progression from the less favorable temperatures to the optimum, there are a number of apparent discrepancies. Some of the cultures became contaminated or the dishes were broken, leaving only one for measurement, as indicated in table 2. On medium 1 certain cultures showed considerably more rapid growth in one dish than in the other, while on medium 2 there may have been a rather wide variation for the three dishes. All serious disagreements between duplicates are also shown in table 2.

TABLE 2.—*Comparison of duplicate cultures showing difference in growth and instances where only one dish was available for measurement at the end of the first or the second week*

Medium and fungus	Temperature	Radial growth of duplicate cultures for the first week		Medium and fungus	Temperature	Radial growth of duplicate cultures for the first week	
MEDIUM 1	°C.	Mm	Mm	MEDIUM 1—continued	°C.	Mm	Mm
Fomes annosus (Europe)	16	9	16.5	*Irpex mollis*	24	[a]	------
Stereum gausapatum		[a]	------	*Polyporus sulphureus*		------	[b]
Coliybia velutipes		4	22.5	*Poria zantha* (sensu Lind)		11.5	[b]
Daedalea ambigua		10	17.5	*Coniophora cerebella* (Europe)		17	14
Fomes marmoratus		4	15	*Ganoderma applanatum*		[a]	21
Hydnum pulcherrimum		[a]	------	*Polystictus abietinus* (*Irpex fusco-violaceus*)	28	[a]	------
Polystictus abietinus (*Irpex fusco-violaceus*)		[a]	------	*Polystictus abietinus*		[a]	------
Polystictus sinuosus		20	27	*Polyporus sulphureus* [c]		35	45
Lentinus lepideus, no. 2		5	15	*Stereum rameale*		[a]	------
Lenzites berkeleyi		9	18	*Trametes pini*		[a]	------
Lenzites striata		11	15	*Daedalea unicolor*	32	[a]	------
Merulius lacrymans (United States)	20	13	17	*Irpex mollis*		[a]	------
Phlebia merismoides		12.5	27.5	*Phlebia merismoides*		[a]	------
Pleurotus ostreatus		8	13.5	*Polyporus maximus*	34	[a]	------
Polystictus hirsutus		17	24	*Irpex mollis*		[a]	------
Polyporus radiatus		8	12.5	*Merulius tremellosus*	40	[a]	------
Polyporus schweinitzii		3	[b] 15	*Lenzites berkeleyi*	42	[a]	------
Polystictus versicolor		21.5	26	*Stereum fuscum*		[a]	------
Schizophyllum commune, no. 1		[a]	------	MEDIUM 2			
Stereum rameale		20	27.5	*Merulius lacrymans* (Europe)	28	[b]	------
Trametes pini		1.5	[b] 5.5				

[a] Only 1 dish available for measurement at end of first week.
[b] Only 1 dish available for measurement at end of second week.
[c] Growth at 2 weeks.

The variations for the 20° and 28° cultures on medium 1 can perhaps be explained by the fact that the medium for one of the pair had a suggestion of yeast growth in the stock flask which necessitated a resterilization for 45 minutes at a pressure of 15 pounds. The agar would not then solidify as firmly as before, and its composition was

[3] MONTEITH, J., JR., and DAHL, A. S. A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE. Jour. Agr. Research 36: 897–903, illus. 1928.

probably somewhat changed. Discrepancies occurred frequently at the upper temperature limits and may perhaps have been partly due to variations in temperature in different parts of the incubator chamber, for it is probable that the variation would be great where considerable heat was applied almost continuously at the bottom, as was done in all the tests run at temperatures near the maximum inhibition point for the more resistant species. Furthermore, as the critical point is approached a slight change in temperature may produce a profound effect on both the rate and character of growth. For this reason it has not always been possible to closely define the maximum inhibition point.

It is possible, though not very probable, that the exposure to daylight necessary for recording growth measurements at the end of the first week may, in certain instances, explain the reduction in growth rate. Fritz,[4] for instance, found daylight inhibitive to the development of *Polyporus schweinitzii* in culture.

The species may be arranged in three general temperature groups: (1) A low-temperature group, growing best at 24° C. and below; (2) an intermediate group, growing best between 24° and 32°; and (3) a high-temperature group, growing best above 32°.

In table 3 the tested fungi are arranged according to their most favorable temperatures for growth on medium 1, starting at the lowest temperature. The optima for medium 2 are given in comparison wherever available, although the order may be different from that for medium 1. The optima are for the most part based on 1 or 2 weeks' growth. The maximum temperature at which growth ceases and the difference between it and the optimum for the different species and cultures are also indicated.

TABLE 3.—*Optimum temperatures for growth and the maximum temperatures which prevent growth of the tested fungi*

LOW-TEMPERATURE GROUP (OPTIMUM 24° C. AND BELOW)

Fungus	Temperature (° C.) affecting growth of fungi on—					
	Medium 1			Medium 2		
	Optimum	Inhibiting	Difference	Optimum	Inhibiting	Difference
Merulius lacrymans (United States)	20	28	8			
Fomes nigrolimitatus	20	32	12	20	32	12
Trametes pini	[a] 20	32	12			
Coniophora cerebella (Europe)	24	[b] 32	8	24	32	8
Fomes annosus (Europe)	24	32	8			
Collybia velutipes	24	32	8			
Coniophora cerebella (United States)	24	[c] 34	10	24	34	10
Fomes officinalis	24	34	10			
Phlebia merismoides	24	34	10			
Polyporus schweinitzii	24	34	10			
Polystictus abietinus (*Irpex fusco-violaceus*)	24	34	10			
Stereum gausapatum	24	38	14			

[a] In order to establish an optimum where it is not clearly defined by the graphs, it has been necessary to take into consideration obvious defects in the experimentation and to make as close an estimate as possible from the data at hand.

[b] Trace of growth noted after the second, third, or fourth week.

[c] Trace of growth at 38° C.

[4] FRITZ, C. W. CULTURAL CRITERIA FOR THE DISTINCTION OF WOOD-DESTROYING FUNGI. Roy. Soc. Canada, Proc. and Trans. (3) 17 (sec. 5): 191–288, illus. 1923.

TABLE 3.—*Optimum temperatures for growth and the maximum temperatures which prevent growth of the tested fungi*—Continued

INTERMEDIATE-TEMPERATURE GROUP (OPTIMUM BETWEEN 24° AND 32° C.)

Fungus	Temperature (° C.) affecting growth of fungi on—					
	Medium 1			Medium 2		
	Optimum	Inhibiting	Difference	Optimum	Inhibiting	Difference
Trametes serialis, no. 1	24	34	10	28	36	8
Merulius lacrymans (Europe)	[a] 26	[b] 32	6	[a] 24	34	10
Merulius silvester (Europe)	[a] 26	34	8	[a] 24	34	10
Ganoderma applanatum	28	34	6	[a] 28	34	6
Hydnum ochraceum	28	34	6			
Poria incrassata	[a] 28	34	6	[a] 28	[b] 34	
Stereum rameale	[a] 28	34	6			
Trametes serialis, no. 2	28	34	6			
Poria xantha (sensu Lind)	28	34	6			
Polyporus radiatus	[a] 28	34	6			
Stereum frustulosum	28	34	6			
Lentinus lepideus, no. 1	[a] 28	36	8			
Merulius himantioides	28	36	8	28	36	8
Polystictus abietinus	28	38	10	[a] 26	36	10
Polystictus versicolor	[a] 28	38	10	28	36	8
Corticium chrysocreas	28	38	10			
Corticium effuscatum	28	38	10			
Fomes subroseus	28	38	10			
Polyporus robiniophilus	28	38	10			
Lentinus lepideus, no. 2	[a] 28	40	12			
Stereum fasciatum	[a] 28	40	12			
Fomes pinicola	28	34	6	30	36	6
Fomes everhartii	28	40	12	30	38	8
Fomes marmoratus	28	40	12	[a] 30	38	8
Merulius tremellosus	[a] 28	42	14			
Polystictus sinuosus	[a] 28	42	14			
Schizophyllum commune, no. 2	28	44	16			
Peniophora gigantea	30	34	4			
Poria subacida	30	34	4			
Polyporus sulphureus	30	36	6	30	36	6
Daedalea ambigua	[a] 30	40	10			
Daedalea unicolor	30	40	10			
Fomes rimosus	[a] 30	40	10	30	40	10
Pleurotus ostreatus	30	40	10			
Polystictus tenuis	[a] 30	40	10			
Polyporus maximus	[a] 30	40	10			
Schizophyllum commune, no. 1	[a] 30	42	12			
Fomes igniarius	[a] 30	42	12	32	40	8
Daedalea quercina	[a] 30	44	14			
Irpex mollis	[a] 30	44	14			
Lenzites berkeleyi	[a] 32	44	12			
Hydnum pulcherrimum	[a] 32	44	12			

HIGH-TEMPERATURE GROUP (OPTIMUM ABOVE 32° C.)

Phlebia strigoso-zonata	[a] 34	40	6			
Fomes annosus (United States)	[a] 34	44	10	34		
Lenzites trabea, no. 1	[a] 34	44	10			
Stereum fuscum	[a] 34	44	10			
Polystictus hirsutus	[a] 34	44	12	36		
Ganoderma lucidum	[a] 34	[b] 46	12			
Lenzites trabea, no. 2	34	[b] 46	12	34		
Lenzites striata	[a] 36	44	8	36		
Panus rudis	[a] 36	([d])	10+	36		
Lenzites saepiaria	36	([c])				

[a] In order to establish an optimum where it is not clearly defined by the graphs, it has been necessary to take into consideration obvious defects in the experimentation and to make as close an estimate as possible from the data at hand.

[b] Trace of growth noted after the second, third, or fourth week.

[d] Above 46° C.

[c] Not determined.

DISCUSSION

It will be noted that the growth of about 85 percent of the species is prevented within a range of 12° above the optimum. The maximum temperature which totally inhibits growth bears no definite relationship to the optimum, but appears to be specific with the organism. However, with one exception, all the species showing a difference of 6° C. or less between the maximum and optimum temperatures have an optimum at 32° or below.

The two media gave different results, as would be expected, but these rarely differed over 2°. In some cases shifting of the maximum temperature has a physiological explanation in variation in the media. Weimer and Harter[5] have shown that the maximum temperature which inhibits growth of *Rhizopus nigricans* Ehr. on potato agar can be raised 1° to 1.5° C. by the addition of definite amounts of dextrose.

Working with *Monilia* and *Rhizopus*, Brooks and Cooley[6] found that *Monilia* grew just as freely at the higher temperatures and earlier and more rapidly at the lower temperatures when developed on peaches than when grown on agar, whereas the reverse condition held for *Rhizopus*. They suggested that this difference might partly be due to the fact that *Monilia* is parasitic and therefore better suited to growth on living material than is *Rhizopus*, which is saprophytic.

In a study involving four fungus parasites of citrus trees, Fawcett[7] found that for three of the fungi the optimum temperature for growth on the same medium usually shifted to lower temperatures for each successive 24-hour observation period during culture periods of from 3 to 6 days. That this happened in certain instances is indicated by a downward shifting of the optimum temperatures in the graphs of 7 of 21 fungi by the end of the second observation period. With the other 14 cultures there was no change in the optimum temperature. There is, however, a limit to the downward shifting of the optima, for if it continued progressively, even at a reduced rate, an organism producing gradual decay, such as heart rot in standing timber, would theoretically attain its best development near the lowest temperatures that permit growth.

This downward shifting of the optimum temperatures suggests that the optima for long-time timber decay would be lower than those indicated here for comparatively short culture periods. There is, moreover, evidence that the progress of wood decay in nature is often slower than that indicated for pure cultures of fungi on agar. For instance, if the decay caused by *Polyporus schweinitzii* developed in the heartwood of a southern pine as rapidly as the fungus grew during its first week in culture at 24° C., by the end of the second year its vertical progress in the tree would be 11.6 feet. Observations on this type of decay in nature point to a much slower rate of development.

The data clearly show the marked inhibiting effect of the lowest temperatures. At 12° C. growth may be entirely inhibited or one half to two thirds retarded; with many of the species it may be

[5] WEIMER, J. L., and HARTER, L. L. TEMPERATURE RELATIONS OF ELEVEN SPECIES OF RHIZOPUS. Jour. Agr. Research 24: 1–40, illus. 1923.

[6] BROOKS, C., and COOLEY, J. S. TEMPERATURE RELATIONS OF STONE FRUIT FUNGI. Jour. Agr. Research 22: 451–465, illus. 1921.

[7] FAWCETT, H. S. THE TEMPERATURE RELATIONS OF GROWTH IN CERTAIN PARASITIC FUNGI. Calif. Univ. Pubs., Agr. Sci. 4: 183–232, illus. 1921.

only 5 to 10 percent as rapid as that at the optimum. It is well known, however, that low temperatures, even those far below zero, do not kill wood-destroying fungi.

A large number of the species studied show very rapid growth over a considerable range of temperature around the optimum; these tolerate a rather high maximum, even as much as 12° to 16° C. above the optimum.

The maximum temperature which inhibits growth is of particular significance when one wishes to determine whether it will be feasible to attempt to control certain of the organisms by the artificial application of heat. Favorable results are more likely to be secured with the low-temperature group, since it is much easier to heat the timber to a point at which the organisms will be killed. The temperatures which can be applied in buildings will depend upon the effectiveness of the heating systems. In buildings having automatic sprinkler systems, the safe maximum temperature is about 46° C. (115° F.).

It has long been recognized by European investigators that the true *Merulius lacrymans* (*Merulius domesticus* Falck) is a comparatively low-temperature form (fig. 1, *A* and *B*), and this opinion is borne out by the present study. It is not unique in this respect, however, since several other species fall in the same class. *Merulius lacrymans* is not a common dry-rot fungus in the United States, although many collections of *Merulius* have been incorrectly referred to this species. So far as known, all of them grow best at temperatures higher than those most favorable for *M. lacrymans*. The growth of this fungus in Petri dishes at various temperatures is shown in figure 4.

When fruiting bodies are absent and it is desired to distinguish between the work of known and unknown fungi both of which cause the same type of decay, the data presented herein may occasionally be of service in identifying the unknown fungus by showing its temperature relationships. For example, while the rot of the American form of *Merulius lacrymans* might be confused with that of *Poria incrassata*, there is enough difference in the temperature relationships of these two fungi to permit ready differentiation in culture.

SUMMARY

In a laboratory study of the effect of temperature on the growth of 64 species and strains of wood-destroying fungi, 12 cultures were found to have optimum temperatures of 24° C. or below; 42, optimum temperatures between 24° and 32°; and 10, optimum temperatures above 32°.

The maximum temperatures for growth were determined for all but two cultures; 62 cultures stopped growth at 46° C. or below; 46 cultures, at 40° or below; and 24 cultures, at 34° or below.

With one exception, all species showing a difference of 6° C. or less between the optimum and maximum temperature had their optimum at 32° or below.

Many of the species showed a rapid growth over a considerable range of temperatures around the optimum, and these tolerated a high maximum temperature, 12° to 16° C. above the optimum.

A downward shifting of the optimum temperatures for growth for successive 7-day observation periods suggests that the

optimum temperatures for growth of wood-destroying fungi under controlled laboratory conditions are in some cases higher than

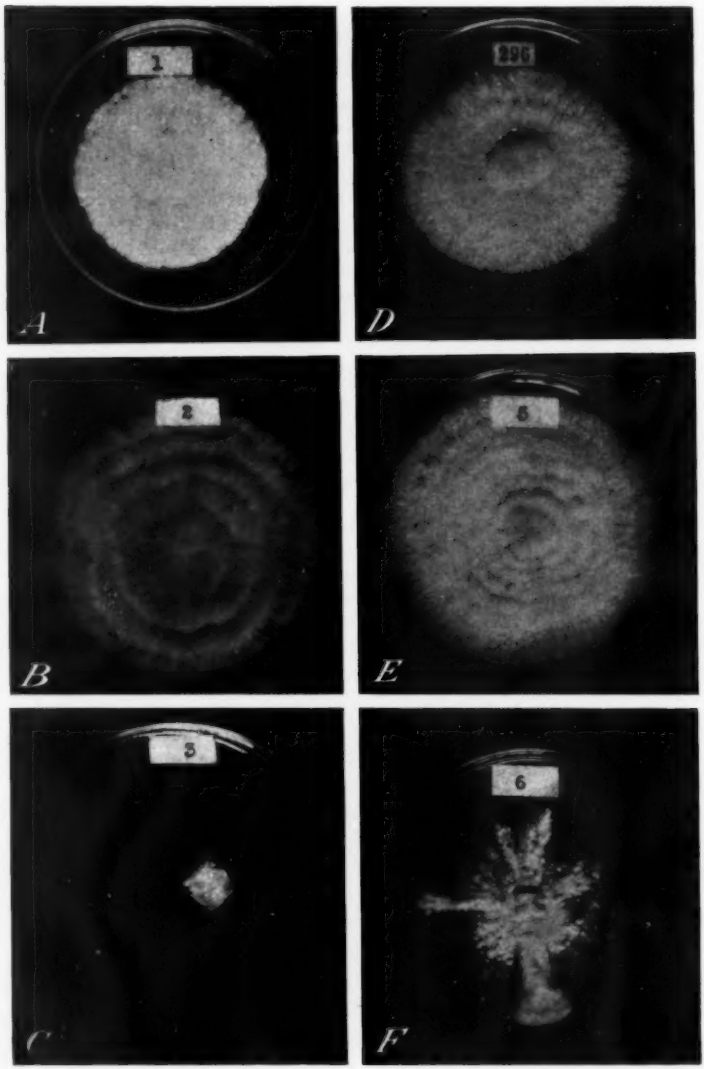

FIGURE 4.—*A-C, Merulius lacrymans* (Wulf.) Fr. (United States), growing on medium 1 (see fig. 1, *A*): *A*, 16 days old at 16° C.; *B*, 15 days old at 20°, its optimum; *C*, 32 days old at 28°. *D-F, Merulius himantioides* Fr., growing on medium 1 (see fig. 2, *A*): *D*, 16 days old at 16°; *E*, 16 days old at 30°; *F*, 31 days old at 32°.

the optima for the same decays in nature when carried over long periods.

SOME DETAILS OF MUSCLE STRUCTURE REVEALED BY SALT EXTRACTION [1]

By H. N. BAKER, *assistant animal husbandman*, HUGH C. MCPHEE, *senior animal husbandman*, and PAUL E. HOWE, *senior chemist, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Studies have been made [2] of factors, in the structure of muscle, which may influence the quality of meat, especially its tenderness. Since the development of an animal involves factors of breeding, feeding, and management, it is necessary to have a technic by which variations in structure due to these factors can be determined. In a study of muscle structure two points are of particular importance: (1) the size and structure of the fibers and (2) the character and distribution of the surrounding connective tissue.

The compactness of muscle tissue often interferes with the microscopical differentiation of the elements of its structure. To facilitate the study of tissue, the writers have used various concentrations of neutral salts, such as sodium chloride, and neutral mixtures of phosphates to remove that part of the protein material which is soluble in these solvents. Examination of finely ground muscle tissue that had been extracted for the determination of the quantity of protein in fresh tissue [3] indicates that although there may be some distortion of the various elements, extraction of muscle with neutral salts does not destroy the essential elements of muscle structure. Studies of extracted frozen sections indicate that the removal of the soluble proteins brings out certain details of structure that are not so evident in the unextracted material.

This paper deals especially with a technic used for the removal of proteins from muscle by sodium chloride and mixtures of monopotassium and dipotassium phosphates followed by microscopic examination of the extracted tissue.

MATERIAL AND METHODS

Muscle tissue from cattle constituted the principal source of material, although the wasp, *Polistes annularis*, was used for studying certain details of structure remaining after phosphate extraction.

Cattle tissue was taken from the muscle having its origin on the symphysis pubis as soon as the blood had drained from the body and before rigor mortis had set in. This method permitted the maximum extraction before some of the protein became denatured.

[1] Received for publication Aug. 19, 1933; issued February 1934. This study was conducted as a part of a national project, cooperative meat investigations, by the Animal Husbandry Division, Bureau of Animal Industry, U.S. Department of Agriculture, and by cooperating State agricultural experiment stations.

[2] BAKER, H. N., MCPHEE, H. C., and HOWE, P. E. THE MEMBRANOUS STRUCTURE OF MUSCLE. (Abstract) Anat. Rec. 41: 64. 1928.

[3] See footnote 2.

The muscle usually was contracting spasmodically when the small cube of tissue was placed on the freezing microtome.

In most cases the sections were cut 25μ in thickness with a freezing microtome. Thin sections were handled by mounting on cellophane. This is accomplished by laying the cellophane on the surface of the cut section as it lies on the microtome knife (fig. 1). Such mounting facilitates the handling of the delicate sections through the various solutions and forms a mount for those sections that are carried through the usual histological procedure. It has also been of value in the further separation of the proteins from the resistant structures of the muscle.

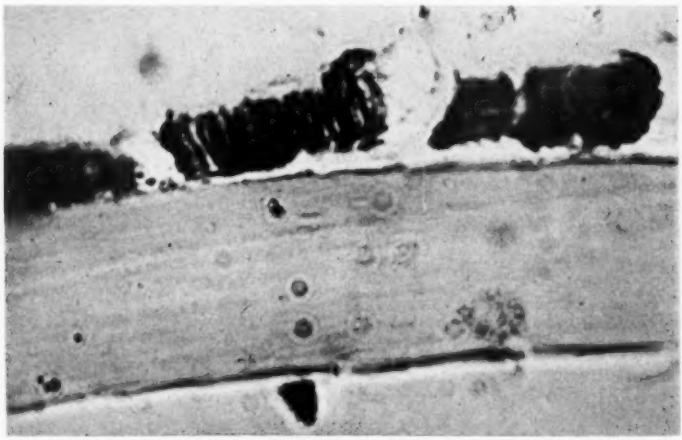

FIGURE 1.—Longitudinal view of fiber in frozen section of beef extracted in a 0.525 molar solution of monopotassium and dipotassium phosphate for 90 minutes and fixed in mercuric chloride, mounted on cellophane, resectioned in paraffin at from 5 to 7μ, and stained with methylene blue. Note individual fibrillae within the fiber and also thickness of cellophane. × 800.

Extractions of the sections of freshly killed tissue were conducted in shallow dishes (half Stender dishes) with concentrations of salt, as suggested by Howe [4] and the subsequent work of Ritchie and Hogan [5] and of Hilberg, Breden, and Howe.[6] Analytical work by Howe [7] has shown that a 0.225 molar concentration of a mixture of monopotassium and dipotassium phosphates having a pH value of 7.4–7.0 gives the maximum extraction, from 40 to 60 percent of the total nitrogen, with these salts. Furthermore, a 0.525 molar concentration of the phosphates extracts practically the same quantity of protein as 0.225. Hilberg, Breden, and Howe [8] have found that a greater extraction of proteins from muscle is obtained with a solution of

[4] HOWE, P. E. THE DIFFERENTIAL EXTRACTION AND PRECIPITATION OF THE SOLUBLE PROTEINS OF MUSCLE, WITH DATA ON THE CONCENTRATION OF THE PROTEINS IN THE MUSCLE OF THE CALF, COW, AND RABBIT. Jour. Biol. Chem. 61: 493–522, illus. 1924.

[5] RITCHIE, W. S., and HOGAN, A. G. THE SEPARATION OF THE SOLUBLE PROTEINS OF RABBIT MUSCLE. Jour. Amer. Chem. Soc. 51: 880–886. 1929.

[6] Unpublished data.

[7] Howe, P. E. (See footnote 3.)

[8] Unpublished data.

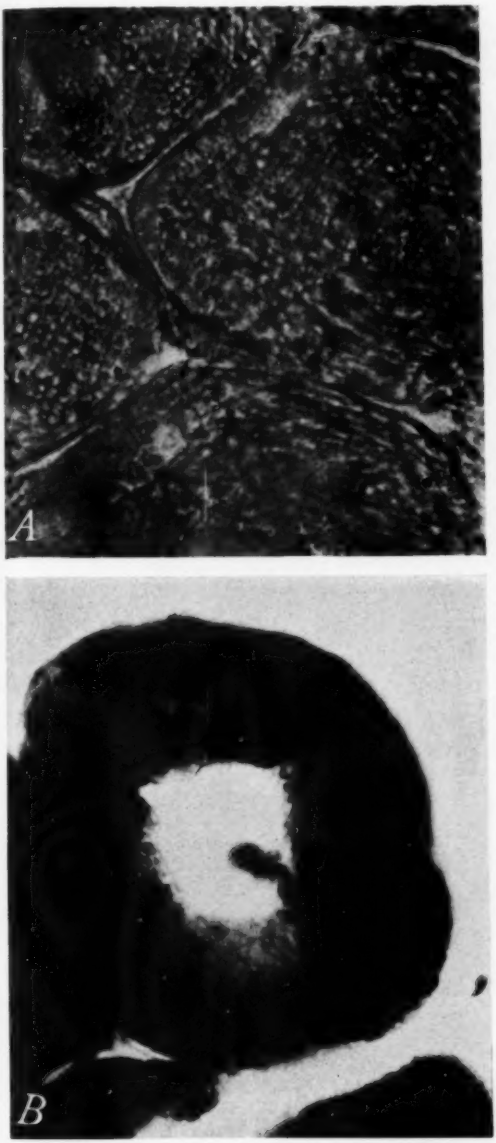

FIGURE 2.—*A*, Cross section of muscle fiber of beef showing slight pits in surface due to dissolving action of water. This is the control for comparison with the sections extracted with sodium chloride. ×1,250. *B*, Muscle fiber extracted with sodium chloride 1.25 molar for 90 minutes. Sections cut at 25μ, stained with methylene blue, and mounted in balsam. Note the sarcolemma or reticular hull. Since this view is not directly down into the fiber, some of the striations may be seen on the side at right angles to the longitudinal plane. ×1,250.

halides than with the phosphates, and that the maximum extraction, roughly from 60 to 80 percent of the total nitrogen, occurs at approximately a 1.25 molar concentration. Most of the work was carried out, therefore, with solutions of 1.25 molar sodium chloride and 0.225 or 0.525 molar mixture of the potassium phosphates.

Extractions were conducted at 15° C. for 1½ hours. The temperature of extraction is not important provided the tissue proteins do not become denatured or insoluble at the temperature used. In the examination of tissue, sections were usually mounted as follows: One section after washing, mounted in glycerin on a glass slide; one section after washing with the same concentration of salt solution, stained in methylene blue and in Scharlach R; one section after washing, fixed with mercuric chloride and stained with Mann's methyl blue and eosin.

RESULTS AND DISCUSSION

The accompanying photomicrographs (figs. 2, 3, and 4) of extracted and unextracted muscle indicate the extent to which muscle struc- is brought out or

modified by extraction. The two kinds of solutions, one containing a halogen salt as represented by sodium chloride and the other consisting of a mixture of phosphates, leave very characteristic pictures, corresponding in part to the degree to which protein has been extracted and in part to the specific effects they have in causing certain structural elements to swell.

The effect of extracting with sodium chloride (1.25 molar solution) is indicated in figure 2, *B*. Figure 2, *A*, a cross section of muscle tissue which has been treated in water, shows that water removes but

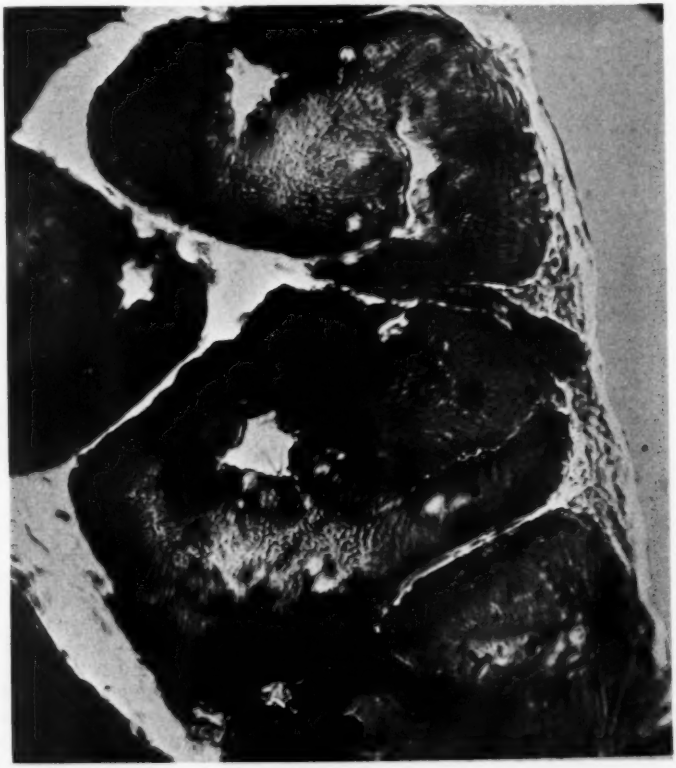

FIGURE 3.—Structural details of muscle fiber after extraction with 0.225 molar monopotassium phosphate and dipotassium phosphate solution. A cross section of beef muscle cut on the freezing microtome at 25μ and then extracted in the salt solution for 90 minutes. Fixed in mercuric chloride and stained with Mann's methyl blue and eosin. Mounted in balsam. × 400.

a small proportion of the soluble protein of muscle. Water also has a precipitating effect upon certain proteins. There is a slight erosion of the muscle. The general effect, however, is not greatly to modify the appearance of the sections.

Sodium chloride solutions in the concentrations used have a very pronounced effect on the appearance of muscle fibers. The fibrils in many cases appear to be completely removed, leaving a much thickened rind. In thin sections the fibers appear as short tubes. Since

fibrils are no longer evident, it appears that the membranes surrounding them consist either of sarcolemma and an inner layer of tissue that readily swells in salt solutions or that the sarcolemma itself becomes

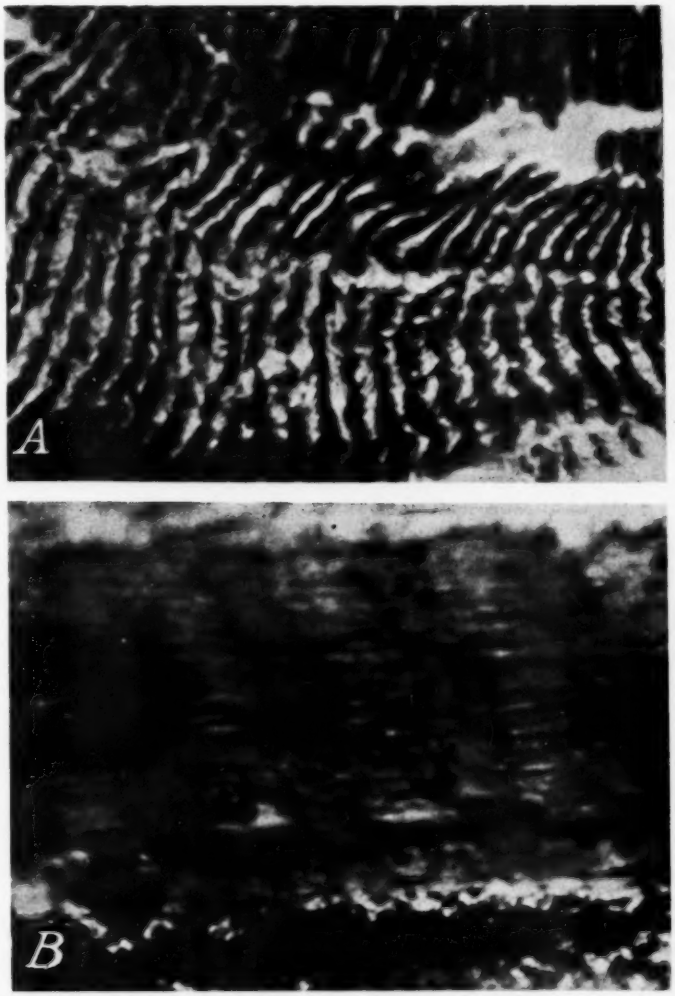

FIGURE 4.—*A*, Longitudinal section of muscle from leg of wasp, after extraction in a 0.525 molar potassium phosphate mixture. Fixed with mercuric chloride, lightly stained with methylene blue, and mounted in balsam. Note depleted nature of muscle fibers. × 2,000. *B*, Longitudinal section of muscle from leg of wasp. No extraction. Used as control. Fixed with mercuric chloride lightly stained with methylene blue, and mounted in balsam. × 2,000.

enormously swollen. This swelling of the muscle tissue, and especially the fiber membrane, in a strong salt solution is characteristic of the halogen salts at the concentrations the writers have used. As indi-

cated before, water has a precipitating effect upon certain muscle constituents. Higher concentrations of salts also cause precipitation. The swollen fiber membrane retains certain structural characteristics. Evidences of cross striation remain, and the outer hull seems to possess uprights or ribs lying close together.

Extraction with a mixture of potassium phosphates having a volume molar concentration of from 0.225 to 0.525 at apparently pH 7.0–7.4, is not so drastic as with the halogen salts (fig. 3). The quantity of protein removed from muscle is, as already indicated, less than that removed by the halogens. Cross sections of fiber have a very characteristic appearance. The fiber usually appears pitted, possibly because of a distortion of the bundles of fibrils in the process of extraction. Most of the supporting structure seems to be present. The ends of the fibrils are present either in the cross section or as frayed ends in the longitudinal section. Examination of a longitudinal section of the muscle of the leg of a wasp (fig. 4) shows that the thin walls of the fibrils are intact and that the material forming the cross striation remains. In some cases the walls of the fibrils appear to be partially collapsed. It would appear, therefore, that fibrils are definite structures from which a considerable quantity of material may be removed without destroying the walls or certain portions of the cross striations. The effect of extraction in bringing out the supporting structures of muscle is clearly indicated by figure 4, *A* and *B*.

Some fibers, the red ones presumably, are not so clear and transparent as the white. In these darker fibers during extraction there seems to be a diffusion of the dark coloring material, probably myohaemoglobin. Fats and lipide material seem to have been trapped in the cells and against the membranes, as shown by the staining with Scharlach R. Staining of the freezing microtome sections brings this out. Not all of it is caught in this way but certainly a considerable part is so retained.

The general effect of extraction may be a clarification of the picture of muscle structure through the removal of soluble proteins, apparently without any considerable damage to the remaining structures, or by the partial removal of some of the fibrillar structures. In either case the connective tissue elements are only slightly affected or are somewhat swollen.

SUMMARY

Studies on muscle from which as much as 60 to 80 percent of the nitrogen had been removed by extraction with neutral salt solutions, showed that the removal of the soluble proteins did not result in any material damage to the remaining structures.

In many cases the fibrils completely disappeared during the sodium chloride extraction, leaving a swollen rind of sarcolemma.

Sections of muscle extracted by sodium chloride and by phosphate mixtures were characteristically different in appearance, and the differences agreed in a general way with the quantity of proteins removed.

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